Application of a clot based assay to measure the procoagulant activity of stored allogeneic red blood cell concentrates

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ABSTRACTS

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Animal, Cellular and Molecular Models

AMC01

Endothelial injury and hypoxia enhances neutrophil recruitment, extravasation, and apoptosis during thrombus formation

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Background: Primary trigger of cardiovascular events remains unclear due to the absence of direct analysis tools of *in vivo* thrombus formation.

Aims: To elucidate blood cell behavior and vascular responses, we improved *in vivo* imaging technique based on multi-photon microscopy, and analyzed new three animal thrombus formation models.

Methods: We combined multi-photon microscope technique, and light-manipulations system, which enabled us to evaluate platelet aggregations by photochemical reactions.

Results: First, we induced rapidly developing thrombi composed of discoid platelets, which was triggered by ROS production without endothelial damage. Discoid platelet activations and aggregations were induced without leukocyte recruitment in this system.

Second, thrombus formation was also induced by direct endothelial cell disruption by femto-second laser irradiations. With the rapid recruitment of inflammatory neutrophils into damaged area, following fibrin net formation and tissue regenerative changes were observed, indicating tight association between endothelial damage and inflammatory reactions, Spontaneous apoptotic neutrophil death, and endothelial-neutrophil- interactions were induced after platelet aggregations.

Last, platelet aggregations, and inflammatory neutrophil recruitment were also observed after transient ischemia and reperfusion. Combination of hypoxia and endothelial injury enhanced thrombus formation, and extravasation steps were remarkably enhanced. Leukocyte escaped from specific and limited "holes" in endothelial junctions.

Conclusions: Intravital visualization of thrombus formation elucidated association of inflammation of endothelium, leukocyte recruitment, and platelet aggregations *in vivo*. Neutrophils were selectively recruited in early phase, which was enhanced by endothelial damages and hypoxic conditions. Our real-time imaging system can evaluate multicellular thrombotic processes and therapeutic strategies against them.

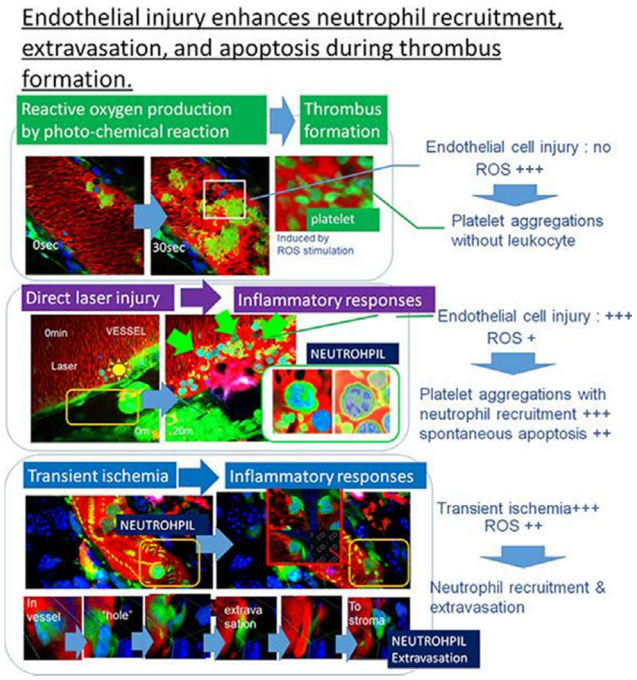

Figure 1

AMC02

TMEM16F-mediated platelet pro-coagulant activity contributes to infarct progression after ischemic stroke

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Background: Transmembrane protein TMEM16F, mutated in patients with the bleeding disorder Scott syndrome, is important for the scrambling of phosphatidylserine (PS) to the platelet surface upon platelet activation. Thus, it plays an essential role in the platelet pro-coagulant response. However, the contribution of TMEM16F and the pro-coagulant activity of platelets to thrombo-inflammation after ischemic stroke is unknown.

Aims: To investigate the pathophysiological role of TMEM16F-mediated platelet pro-coagulant activity in the setting of ischemic stroke.

Methods: A platelet and megakaryocyte specific TMEM16F knockout (KO) mouse was generated by targeted deletion of exon 3 in the *Anoctamin6* gene. The mice were assessed in the transient middle cerebral artery occlusion (tMCAO) model of ischemic stroke in addition to models of thrombosis and hemostasis, as well as *in vitro* platelet analyses.

Results: TMEM16F KO platelets exposed significantly less PS and also failed to acquire a ballooned morphology after stimulation with ionomycin, indicating a reduced pro-coagulant potential. Likewise, thrombinoscope measurements showed that time to peak thrombin concentration was significantly delayed in KO platelet rich plasma (PRP), as compared to WT PRP. KO mice displayed significantly prolonged tail bleeding times, and were protected in a model of ferric chloride induced thrombosis in the carotid artery, confirming previous reports. These results highlighted the TMEM16F KO as a suitable model with which to investigate the pathophysiological significance of pro-coagulant platelets in cerebral infarct progression following tMCAO. Initial experiments indicate that female TMEM16F KO mice are protected from infarct growth after tMCAO. Further experiments are underway to test if this is also the case in male mice and to understand how pro-coagulant platelets contribute to thrombo-inflammation during ischemic-reperfusion injury.

Conclusions: These results add to the current understanding of the role of platelets in thrombo-inflammation.

AMC03

Time-dependent decay of mRNA, ribosomal RNA and translation activity in platelets

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Background: Experiments performed during the first decade of this century have suggested that translation in platelets could be biologically important. For example, LPS was shown to induce IL-1beta synthesis in each platelet. Nevertheless, long RNA molecules appear to be mostly present in the minor population of the youngest platelets. In addition, the amount of RNA in platelets is hardly compatible with a protein synthesis in all these cells.
Aims: To attempt to solve this paradox, we studied the time-dependent changes of RNA molecules present in platelets in terms of amount, integrity and biological activity.
Methods: We administrated diphtheria toxin in mice expressing its receptor in megakaryocytes for 4 days, provoking a strong thrombocytopenia followed by dramatic thrombocytosis characterized by a short period with a vast majority of a few hours old reticulated platelets. The fates of ribosomal and/or messenger RNAs in these cells were analyzed using *ex vivo* and *in vivo* chase experiments.
Results: Experiments indicated that the RNA content of freshly isolated reticulated-enriched platelet populations was 20 higher than that of control normal platelets. *In vivo*, they lose most of their RNA within 12 h. *Ex vivo* at 37°C, their total RNA content decreased a twofold within 6 h while their integrity was compromised. Accordingly, fluorescence *in situ* hybridization techniques confirmed the presence of beta actin mRNAs in most of reticulated-enriched platelets (>80%), but detected them in only a minor subset of control platelets (< 10%). *In vitro*, constitutive translation decreased considerably within < 6 h. The RNA content of platelets in transfusion bags also rapidly decayed at 37°C.
Conclusions: Our analyses question how or whether protein synthesis in platelets, especially in non-reticulated ones, could have a biological function *in vivo*. This study points to the importance to take into account quantitative analyses in order to fully appreciate the meaning of biological processes.

AMC04

Platelets promote MMP-dependent plaque destabilization locally at the site of vascular injury

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Background: Platelets are increasingly being implicated in subacute processes, such as atheroprogression. Evidence for this is predominantly based on animal models. A major caveat of these models is that an overt thrombotic trigger, such as occurs in man upon plaque rupture, is either lacking or thrombosis is being triggered in a terminal way.
Aims: To develop a model for studying post-thrombotic vascular remodelling.
Methods: Thrombocytopenia was induced in $Apoe^{-/-}$ mice by injection of αCD42b antibodies. Plaque rupture was induced by temporary ligation of the carotid artery of thrombocytopenic and control-antibody treated normopenic $Apoe^{-/-}$ mice. Matrix degrading activity (MMPsense 680) was monitored non-invasively 24 h after ligation using the eXplore Optix imaging system. Two weeks after ligation, mice were sacrificed and vascular changes were assessed by immunohistochemistry (haematoxylin and eosin, sirius red).
Results: Temporary ligation proximal of the carotid artery bifurcation led to reproducible formation of non-occlusive platelet-rich thrombi that remained present for a prolonged time (intravital microscopy and histology). Locally at the site of ligation, normopenic mice in comparison to thrombocytopenic mice displayed.
I) increased matrix degrading activity (+114%, $P = 0.01$);
II) a thinner fibrous cap (-47%, $P = 0.01$), increased necrosis (+58%, $P < 0.005$), increased proliferation (+95%, $P < 0.05$);
III) medial thickening (+56%, $P < 0.005$) with a trend towards lower collagen burden (−47%, $P = 0.13$).
Conclusions: We established a reproducible model for assessing post-thrombotic vascular remodelling in $Apoe^{-/-}$ mice. Using this model we provide first-time evidence that platelet-thrombi exert persistent matrix degrading activity. The platelet-dependent fibrous cap thinning and increased necrosis at the site of injury point towards a plaque destabilization role for platelet-thrombi. These findings are relevant for the secondary prevention of arterial thrombosis.

AMC05

Genetic or pharmacologic depletion of platelet serotonin attenuates myocardial ischemia reperfusion injury

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Background: The inflammatory response during reperfusion after myocardial infarction leads to neutrophil migration into cardiac tissue, which contributes to deleterious ischemia/reperfusion (I/R) injury. Serotonin is a mediator of neutrophil recruitment and inflammatory response is decreased in mice lacking peripheral serotonin (tryptophan hydroxylase 1 (Tph1)-/-).
Aims: Evaluate the impact of platelet serotonin on neutrophil invasion during I/R injury.
Methods: Myocardial ischemia was induced for 30 min in C57Bl/6 (WT), WT mice chronically treated with the selective serotonin reuptake inhibitor (SSRI) fluoxetine, and $Tph1^{-/-}$ mice, followed by 24 h of reperfusion. Platelet neutrophil complexes (PNC) in infarct tissue and blood as well as several integrins and selectins on circulating neutrophils and platelets were analyzed by flow cytometry. Infarct size was determined using monolite blue/TTC staining and heart function was evaluated by echocardiography.
Results: Echocardiography showed better heart function in Tph1-/- and SSRI-treated mice compared to WT after surgery corresponding to a significant reduction in infarct size (38 ± 3 in Tph, 40 ± 2 in SSRI and 55 ± 2 in WT; %AAR). We found a decreased expression of several adhesion molecules on neutrophils in TPH1-/- mice as well as a weaker activation state of platelets in terms of surface integrins compared to WT mice (Activated GPIIb/IIIa (220 ± 8 vs 260 ± 7), GPIbα (1873 ± 211 vs 2628 ± 160); MFI).
This was accompanied by a reduced amount of circulating PNCs in TPH1-/- and SSRI treated mice. Neutrophil and PNC infiltration into the area at risk was educed by approximately 50%.
Conclusions: Genetic or pharmacologic depletion of platelet serotonin improves the outcome after myocardial I/R injury with better preserved heart function. Serotonin influences neutrophil adhesion and migration and the activation state of platelets, resulting in PNC formation and infiltration. This may open new possible strategies to control the inflammatory aspect of reperfusion injury.

AMC06

Lack of endothelial nitric oxide synthase (eNOS) induces a pro-thrombotic phenotype in mice

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Background: Mice genetically deficient of eNOS, the constitutive enzyme producing NO in the endothelium and platelets, have endothelial dysfunction, hypertension and cardiac dysfunction, but their thrombotic phenotype is controversial and it has not been fully explored.
Aims: Aim of our study was to clarify the role of NO, and in particular of platelet-derived NO, on both arterial and venous thrombosis.
Methods: $eNOS^{-/-}$ mice and wild type (WT) mice made thrombocytopenic and then transfused with $eNOS^{-/-}$ platelets were used. Pulmonary thromboembolism was induced by i.v. injection of increasing doses of collagen+epinephrine (coll+epi); arterial thrombosis was induced by photochemical damage of increasing intensity to the femoral artery; inferior vena cava thrombosis was induced by partial flow restriction.
Results: Platelets from $eNOS^{-/-}$ mice showed enhanced adhesion to collagen at high shear rate *ex vivo*. *In vivo* platelet activation induced by i.v. coll+epi was significantly higher in $eNOS^{-/-}$ than in WT mice (38 ± 4 vs $25 \pm 6\%$ of P-Sel positive platelets, $P < 0.05$). The dose of i.v. coll+epi required to produce ~85% mortality was significantly lower in $eNOS^{-/-}$ than in WT mice. Photochemical injury to the femoral artery led to an occluding thrombosis in a significantly shorter time (7.7 ± 0.3 min vs 11.9 ± 0.7 min, $P < 0.05$) and with a thrombus weight significantly higher (0.59 ± 0.02 vs 0.27 ± 0.01, $P < 0.05$) in $eNOS^{-/-}$ mice. Similarly, WT mice with only circulating platelets lacking eNOS showed a significantly shorter time to occlusion (8.2 ± 0.4 min, $P < 0.05$ vs control mice). Finally, inferior vena cava thrombus weight was significantly enhanced in $eNOS^{-/-}$ mice (16.4 ± 12.3 mg vs 11 ± 2.5 mg, $P = 0.02$).
Conclusions: Altogether our data confirm that endogenous NO exerts an antithrombotic activity *in vivo* both in the arterial and venous bed and that platelet-derived NO contributes to this effect.

AMC07

Microparticles derived from ovarian cancer cell line contained genomic and biologically active proteins, including tissue factor involved in coagulation

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Background: The microparticles (MPs) are involved in the thrombotic process, particularly in cancer.
Aims: Analysis of *in vitro* generation of MPs from unstimulated human ovarian adenocarcinoma cell line (OVCAR-3) or stimulated with activated protein C.
Methods: The MPs present in the supernatant of OVCAR were isolated by ultracentrifugation and were analyzed by flow cytometry, electron microscopy, cryofracture analysis, DNA and RNA, and proteomic analysis. The level of tissue factor (TF) on MP was evaluated by TF-induced shortening of $Ca2^{+}$ plasma coagulation time.
Results: Our results demonstrated that
1) 92% of MPs derived from OVCAR-3 were < 100 nm.
2) As tested by flow cytometry, the MPs contained b2 microglobulin, annexin, DNA fragments and TF responsible for a shortening of Ca^{2+} −induced plasma coagulation time. When OVCAR-3 was cultured for 18H with APC, MPs were generated in greater amount than those generated by OVCAR-3 in its absence and their level of TF was increased of 20%. But, in contrast with intact OVCAR-3 cells, the endothelial protein C receptor (EPCR) was not detected in MPs,
3) Proteomic analysis show that the MPs contain proteins involved in cancer progression such as mucins, keratin type-1, actin, annexin (A1, A2, A4), CD44, glypican, heat shock (70 kDa and HS90α) proteins, Agrin, Ephrin type A receptor, coronin-1C, catenin α, integrin β-1 and also p-selectin responsible of platelet activation. They also express several DNA associated proteins including transcription factors, nucleases, and histones involved in chromosome packaging and transcription in the cell nucleus.
Conclusions: MPs expressed several biologically active proteins, DNA and their associated proteins. They expressed procoagulant TF activity already found on intact ovarian cancer cells and this activity was at a greater extent in the presence of APC, despite the absence of EPCR expression on MPs that was expressed on intact OVCAR-3 cells.

AMC08

Hemostatic disturbances and oxidative stress in experimental *Bothrops jararaca* envenomation: modulation by the natural antioxidant quercetin-3-rutinoside (Rutin)

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Background: Snakebites are considered a major public health issue in Brazil. *Bothrops* envenomation causes thrombocytopenia, hypofibrinogenemia and increased plasma levels of tissue factor (TF). Recently, patients bitten by *Bothrops* snakes were reported to develop systemic oxidative stress, which was not neutralized by the specific antivenin. Therefore, it is important to search for other complementary therapies, such as use of rutin, a natural antioxidant that combats oxidative stress and inhibits protein disulfide isomerase (PDI).
Aims: (a) To characterize hemostatic disturbances, oxidative stress, and protein expression of TF and PDI in mice injected with *B. jararaca* venom (BjV);
(b) to evaluate the possible modulatory activity of rutin on these parameters.
Methods: Swiss mice were divided in four groups (n = 5-10/group) and injected s.c. with: saline (group #1), rutin (14.4 mg/kg b.w., group #2), BjV (1.6 mg/kg b.w., group #3) or BjV+rutin (group #4). After 6 h, in order to determine complete blood cell counts and to assay fibrinogen and reactive oxygen species (ROS), blood was obtained by puncture of caudal vena cava; in order to determine the protein expression of TF and PDI in skin, fragments from the site of injection were excised.
Results: Counts of white blood cells, red blood cells, and platelets, as well as fibrinogen levels, decreased in envenomed groups (#3 and #4) compared to controls (#1 and #2). Plasma ROS levels increased in group #3 compared to other groups. Protein expression of TF increased in group #3 compared with group #1, but PDI showed no statistically significant difference among groups.
Conclusions: In our model, we observed hemostatic disturbances characteristic of *B. jararaca* envenomation, and increased ROS levels that are one parameter indicative of systemic oxidative stress. In this regard, the use of rutin restored ROS levels, acting as a promising antioxidant that may be used as ancillary treatment for *Bothrops* snakebites.

AMC09

Expression of heparanase in cancer as biomarker of malignancies: overexpression in an aggressive, poor survival gastric cancer "gastric signet ring cell carcinoma" compared with that of other gastric cancers

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Background: Gastric signet ring cell carcinoma (GSRC) is a distinct entity among of other gastric cancer characterized by a poor prognosis a low sensitivity to chemotherapy and a high level of fibrosis in the tumor.
Aims: We studiedi ts expression in plasma and cancer tissues from GSRC patients and several cancer cell lines.
Methods: HPA was tested in several cancer cell lines from ovaries (OVCAR-3, SKOV-3), breast (MDA231, MCF7) colon (LS-174), lung (A549), uterus (HELA) and gastric (adenocarcinoma (AGS) and GSRC (KATO-III) using several techniques such as RT-PCR, Q-PCR, immunocytochemistry, flow cytometry and ELISA. The amount of HPA mRNA in the biopsy simples of gastric adenocarcinoma (n = 10) and GSRC (n = 11) in tumors and their environment were analyzed.
Results: A) *HPA gene is expressed in all cancer cell lines, but its level varies depending on the tumor cell line*. In biopsies of gastric cancer, the HPA gene is more expressed in the tumor regions (P = 0.0002) and in tumor environment (P = 0.015) in GSRC than in other gastric adenocarcinoma.
B) *The level of HPA, evaluated by immuno-enzymology show that in the supernatants of 10^6 cancer cells*: the level of heparanase was very high in OVCAR-3 supernatant and in KATO-III cell supernatants.
Conclusions: Heparanase was found in in many cancer cell lines and its level depends on origin of tumor cells and on its aggressivity. Taking into account the pro-metastatic functions, proangiogenic and procoagulant activity of HPA and its overexpression in gastric signet ring cell carcinoma of poor prognosis and its cell line, HPA can be considered as a biomarker of malignancy and as a therapeutic target in GSRC patients.

AMC10

Acquired von willebrand syndrome evoked by experimental *Bothrops jararaca* envenomation

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Background: *Bothrops jararaca* snakebites cause a systemic derangement in hemostasis. Thrombocytopenia, platelet dysfunction and mucous bleeding are observed in patients. Symptoms and laboratorial findings are similar to those of acquired von Willebrand Syndrome (AvWS).
Aims: Due to various clinical factors that might interfere with the analysis of von Willebrand factor (vWF) in patients, we used rats as a model to address whether AvWS does occur during *B. jararaca* envenomation. We also evaluated whether snake venom metalloproteinases (SVMP) are involved in such changes.
Methods: Crude *B. jararaca* venom (BjV) was incubated with saline (positive control) or Na_2-EDTA-treated BjV, and administered s.c. into Wistar rats; negative controls were injected with saline. Blood samples were collected 3 h after injection to evaluate: vWF antigen (vWF:Ag) and activity (vWF:CB), and the ratio between them (CB/Ag ratio); plasma vWF multimer analysis; and platelet counts and plasma fibrinogen levels.
Results: vWF:Ag and vWF:CB were reduced in animals treated with BjV compared with animals treated with saline or Na_2-EDTA-treated BjV. CB/Ag ratio was similar in all groups, once reduction in vWF:CB and vWF:Ag values were proportional. vWF multimer analyses showed a concomitant reduction in high molecular weight multimers and an increase in low molecular weight multimers in BjV-treated animals. On the other hand, Na_2-EDTA-treated BjV showed normal vWF multimer pattern, similar to that of animals treated with saline. Hypofibrinogenemia associated with thrombocytopenia was observed in the saline-treated BjV group. As expected, the Na_2-EDTA-treated BjV group did not show hypofibrinogenemia, but only thrombocytopenia.
Conclusions: *Bothrops jararaca* envenomation causes a reduction in plasma vWF antigen and biological activity, and modifies vWF multimer pattern. Such alterations are similar to those observed in AvWS. Moreover, SVMP seem to be crucial for vWF proteolysis and hypofibrinogenemia. Financial support FAPESP.

AMC11

Patagonfibrase modifies protein expression of tissue factor and protein disulfide isomerase in rat skin

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Background: Patagonfibrase is a hemorrhagic metalloproteinase isolated from the venom of the South American rear-fanged snake *Philodryas patagoniensis*, and is an important contributor to local lesions inflicted by this species. The tissue factor (TF)-factor VIIa complex, besides triggering the coagulation cascade, has been demonstrated to be involved in inflammatory events.
Aims: Patagonfibrase is a hemorrhagic metalloproteinase isolated from the venom of the South American rear-fanged snake *Philodryas patagoniensis*, and is an important contributor to local lesions inflicted by this species. The tissue factor (TF)-factor VIIa complex, besides triggering the coagulation cascade, has been demonstrated to be involved in inflammatory events.
Methods: Patagonfibrase (60 μg/kg) was administered s.c. to rats, and after 3 h blood was collected to evaluate hemostasis parameters, and skin fragments close to the site of injection were taken to assess TF and PDI expression.
Results: Patagonfibrase did not alter blood cell counts, plasma fibrinogen levels, or levels of TF activity in plasma. However, by semiquantitative Western blotting, patagonfibrase increased TF expression by 2-fold, and decreased PDI expression by 3-fold in skin samples. In agreement, by immunohistochemical analyses, prominent TF expression was observed in the subcutaneous tissue.
Conclusions: Patagonfibrase affects the local expression of TF and PDI without inducing any systemic hemostatic disturbance, although evidencing that they may be involved in the inflammatory events induced by hemorrhagic metalloproteinases. Once antivenom therapy is not totally effective to treat the local injury induced by snake venoms, modulation of the activity and expression of TF and/or PDI might become a strategy for treating snake envenomation.

AMC12

iAlgogenic compounds found in rat platelet releasate

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Background: In a previous report, we showed that whole platelets or platelet releasate (PR) from platelet granules caused an increased pain threshold when injected intraplantarly (i.pl.) in rats (Yamashita et al., JTH, 9:2057, 2011).
Aims: To characterize the compound(s) found in PR that are involved in hyperalgesia, and to understand thereby the role of platelets in the pathogenesis of pain.
Methods: Blood from anesthetized male Wistar rats was obtained by puncture of the abdominal aorta and collected in ACD. Washed platelets were stimulated by thrombin to obtain PR, which was then centrifuged and frozen. The molecular mass of algogenic compounds was analyzed by ultrafiltration (nominal molecular weight limit: 3 kDa) of PR. The hyperalgesic response from the supernatant (SP) and ultrafiltered samples (UF), as well as from PR (positive control), was evaluated. To test the involvement of ATP and ADP present in PR in hyperalgesia, ultrafiltered PR was incubated with commercial *Crotalus* phosphodiesterase (PDE) at 37°C for 1 h. To evaluate hyperalgesia, rats were submitted to the paw pressure test after i.pl. injection (100 μL) of samples in hind paws.
Results: UF samples induced hyperalgesic response up to 4 h, similarly to PR. On the other hand, SP failed to induce hyperalgesia or analgesia in rats. These data demonstrate that compounds with molecular mass < 3 kDa account for the algogenic activity of platelets. When the UF samples were frozen, or frozen and lyophilized, no hyperalgesic response was noticed, demonstrating that freezing *per se* destroyed this biological activity. After the incubation with PDE, UF kept its hyperalgesic activity; PDE alone did not induce hyperalgesia.
Conclusions: In this study, we showed that freezing-sensitive small compounds (< 3 kDa) are responsible for hyperalgesia in rats. Despite the presence of adenylated nucleotides in PR, which are important for hemostasis, ATP and ADP secreted from platelets do not seem to trigger the hyperalgesic response.

AMC13

Variegin: pharmacokinetics and pharmacodynamics

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Background: Anticoagulants are used for treatment of thromboembolic disorders. Although effective, anticoagulants such as heparin have many limitations, for example a narrow therapeutic window. This provides impetus for the development of effective anticoagulants without these limitations. Previously, we isolated the variegin peptide from salivary glands of the tropical bont tick (*Amblyomma variegatum*) and shown it to be a high affinity direct thrombin inhibitor (~300 pM) and a potent anticoagulant *in vivo*. Our plan is to develop variegin into a new anticoagulant.
Aims: (1) Efficacy and safety comparisons of variegin to unfractionated heparin in animal models.
(2) Pharmacokinetics (PK) studies of variegin using LC-MS/MS.
Methods: Drug administration: The left femoral vein and artery of each rat was cannulated for drug administration and blood collection, respectively. Rat carotid artery thrombosis model: A $FeCl_3$-soaked filter paper was placed on the carotid artery to induce thrombosis. A doppler flow probe was used to measure time-to-occlusion (TTO). Thrombus was dissected out and weighed. Rat tail incision model: A 5 mm incision is made on the ventral side of the rat's tail. Blood was dabbed from the side of the wound using filter paper. Bleeding time was measured. PK studies: Variegin was administered to rats at doses of 0.25, 0.5, 1 and 2 mg/kg. Blood samples were collected over-time. LC-MS/MS was used to quantify variegin in plasma.
Results: Increasing doses of variegin and heparin resulted in increased TTO and corresponding decrease in thrombus weight. Furthermore, doses of variegin that resulted in a similar antithrombotic effect (TTO) in comparison to heparin conferred lower bleeding times. Using non-compartmental analysis, the elimination half-lives for 0.25, 0.5, 1 and 2 mg/kg variegin were 32.3 ± 3.0, 37.9 ± 4.6, 41.3 ± 14.5 and 36.5 ± 13.3 min, respectively.
Conclusions: Variegin has a larger safety window in comparison to unfractionated heparin. PK parameters for variegin were obtained using LC-MS/MS.

AMC14

Aminoestrogen diebud decreases circulating microparticles

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Background: A pro-thrombotic prone condition increases with the use of estrogens, mainly in FV Leiden carriers, also the circulating microparticles (Mps) increases during the hormonal replacement therapy.Mps are involved in numerous biologic functions (physiological homeostasis, inflammation, coagulation, etc.), and could be a possible link between a pathological condition and the vascular homeostasis.
Aminoestrogens, compounds designed and synthetized in the search of a estrogenic therapy without a thrombotic secondary effect, increases the nitric oxide production and decreases platelet aggregation.
Aims: Determining Mps circulating concentration in mice treated with the aminoestrogens, Buame and Diebud.
Methods: The estrogens compounds were synthesized at Instituto de Química, UNAM. Male adult CD1 mice weighing 30–40 g were kept under controlled temperature and light-dark cycles of 12 h. Food and water were given *ad libitum*. Estrogens compounds (Estradiol, Buame and Diebud) were dissolved in dimethylsulfoxide (DMSO). After 8 days of pre-treatment (3 mg/100 g body weight, subcutaneously) or DMSO (control group), a sample of blood was obtained under anesthesia. For each assay, blood was immediately anticoagulated with citrate. Mps were obtained by ultracentifugation followed by a thermic shock. The concentration of protein was determined and related to a calculated Mps concentration following a formula developed by Freyssinet and Toti (1 μg/mL protein=$8x10^5$MP/μL). Samples were stored at -80°C until assayed.
The protocol of this study followed all the ethical and legal regulations outlined for animal experiments and was approved by the ethical institutional committee.

Results:

Table 1 Circulating microparticles under aminoestrogens treatment.

Group	*N*	MP concentration (x 10^7MP/μL)
Control (V)	5	5.5 (3.4–10.6)
Estradiol (E2)	8	11.0 (3.8–43.8) *
Buame (B)	6	5.0 (0.9–12.9)
Diebud (D)	6	2.9 (2.2–4.6) *

Median (minimum-maximum); Kruskal Wallis with U de Mann Whitney; * $P < 0.05$ was considered significate statistically. SPSS v21.0.

Conclusions: Mps circulating concentration increases twice with estradiol, Diebud instead decreses 2.8 times the circulating microparticles. Buame has no effect.

Biorheology

BR01

Microfluidic platform for automated endothelial cell culture enabling real time visualisation of blood cell function under flow

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Background: Microfluidic research platforms can allow precise control of experimental parameters.
Aims: Our aim is to generate a microfluidic platform facilitating *in vitro* cell culture and flow based experimentation. The platform should allow high-resolution microimaging of adherent co-cultures, while enabling high temporal resolution drug and reagent delivery, with real time monitoring of paracellular permeability via measurement of electrical resistance. The platform should allow for significant automation of cell culture conditions, and on-chip experimental control for microscopy-based experiments.
Methods: The microfluidic device is fabricated using a novel 'injection moulding' fabrication technique. A porous membrane is embedded between micro-channels, providing a co-culture platform (Figure 1a). This platform is used to observe the influence of drugs on cell cultures, as well as interactions of suspended blood cells and blood components with adherent cell cultures, under well-defined conditions. On-chip valves can allow rapid delivery of drugs and reagents, to active cultures with high temporal resolution (Figure 1b).
Results: As a proof-of-concept demonstration of the platform, we emulate the Blood Brain Barrier (BBB) through co-culture of primary human brain microvascular endothelial cells and Human transformed SVG astrocytes. Cell morphology change under application of tissue plasminogen activator (tPA) was monitored using phase contrast microscopy, in combination with real-time TEER measurements (Figure 1c). More complex intercellular and intracellular events can be monitored using high-resolution epifluorescence and resonant scanning confocal microscopy.
Conclusions: This research platform has been applied as a simplified *in vitro* model of the BBB. Future studies are aimed at delineating the way in which BBB integrity is modified in response to tPA and investigation of the way macrophage and platelet adhesion and transmigration dynamics are effected by a novel series of single chain antibody-CD39 fusion constructs.

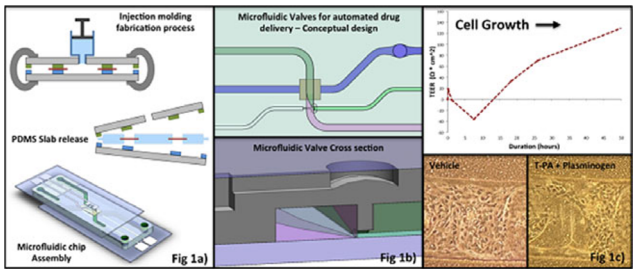

Figure 1

BR02

Occlusive thrombus growth at high shear rates: comparison of whole blood and platelet rich plasma at constant pressure

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Background: Bulk thrombosis in stenotic arteries show rapid growth of platelets-to-platelets different from the static phase of platelet adhesion to the surface. Rapid accumulation to flow occlusion may or may not be affected by RBC, depending on the hemodynamic conditions.
Aims: We explored the role of driving pump (gravity vs. syringe) and hematocrit on the growth rate of bulk thrombus.
Methods: Platelet Rich Plasma (PRP) was separated from porcine Whole Blood (WB) by gravity to prevent platelet activation. Blood was perfused through a stenotic microfluidic chamber of 82 μm height coated with fibrillar collagen to study bulk thrombosis. Perfusion under gravity created a constant pressure head to yield wall shear rates of 3500–6000 s^{-1} in the stenotic region across 90% of the width. Microscope images and optical intensities were analyzed.
Results: The intensity of the image changed over time during gravity perfusion. After the occlusion, the intensity inflected as thrombus consolidated. The occlusive thrombus showed multiple, persistent channels with fluid motion (10 μm/s) after the net bulk flow stopped. The channels were about 20 microns in diameter and yielded a pore fraction of about 20% (Figure 1). Lag time was slightly slower for PRP compared to WB seen previously with syringe flow (Figure 2), suggesting that surface adhesion depends primarily on vWF absorption. In contrast, the occlusion time was longer for PRP compared to WB with gravity driven flow. Thus, RBC play a significant role for thrombus growth, causing platelet margination and increase in effective diffusivity promoting more rapid accumulation.

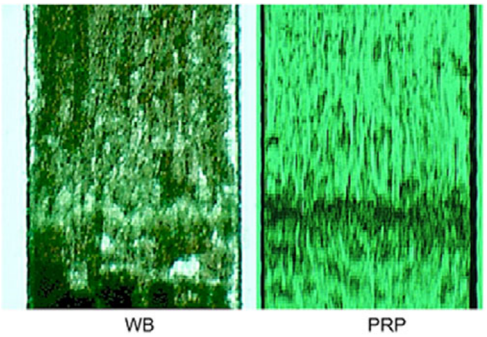

Fig. 1 – Microscopic image near occlusion showing channel pores and RBCs.

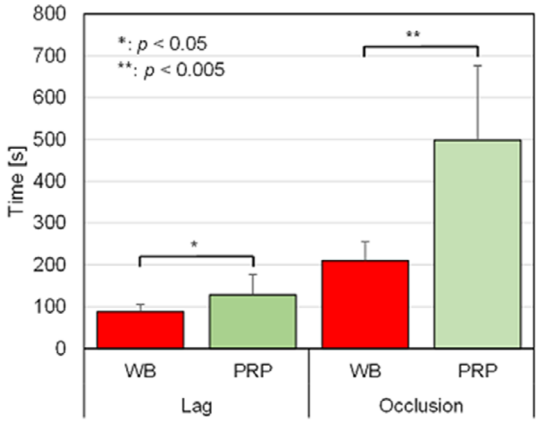

Fig. 2 – PRP growth is much slower with 2.4X longer occlusion time than WB with gravity driven flow, likely reflective of the 2.4X platelet concentration increase with margination.

Conclusions:

1. Bulk thrombus has significant pores at mass occlusion with velocities of 10 μm/s. Thrombus intensity changes dramatically after thrombus consolidation.
2. Thrombus growth slows with PRP for constant pressure as shear remains moderate, in contrast to more rapid PRP growth with a syringe pump where shear rates go much higher.

BR03

Shear-dependent red blood cell and platelet adhesion in sickle cell disease

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Background: Biophysical characteristics of red blood cells and platelets play a critical role in vascular occlusions that lead to life-threatening crises and complications in sickle cell disease. Sickle erythrocytes display reduced cellular deformability and increased adhesion onto (sub)-endothelium matrix proteins such as Laminin (LN) and Fibronectin (FN).
Aims: We present a physiological microfluidic approach to create a shear gradient along the flow direction to investigate the adhesion of sickle Red Blood Cells (sRBC) and platelets under continuously transitioning shear rates. We hypothesized that sRBC subpopulations have distinct adhesive characteristics, and platelets play a role by increasing the adhesion affinity of RBCs.
Methods: We utilized a microfluidic platform to assess sRBC and platelet adhesion using 18 whole blood samples from individuals with hemoglobin SS, and quantified shear dependent adhesion properties of sRBCs and platelets.
Results: We observed greater adhesion of sRBCs at lower shear sites. Deformable cells displayed the highest rate of shear dependent adhesion to LN. Non-deformable cells adhered to FN were the least shear-influenced group that often required greater shear rates over the physiological range for complete detachment. We observed that platelet mediated sRBC adhesion affinity is up to four times greater than sRBC adhesion directly to FN.
Conclusions: These data suggest a heterogeneous shear dependency of sRBC subgroups, and a potential role of platelets in mediating sRBC adhesion to endothelium. Utilizing this microsystem, it's possible to investigate the effect of shear stress on various biological flow conditions, such as the interplay between the shear rate, platelet activity, thrombosis, and haemostasis in sickle cell patients.

BR04

Clot-bound thrombin under venous flow conditions

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Background: In closed model systems of fibrin formation, exosite mediated thrombin binding to fibrin has been characterized as low affinity but high availability. This pool of active thrombin has been hypothesized to contribute to clot stability.
Aims: To assess the stability and accessibility of fibrin bound thrombin at various fibrin densities under venous flow conditions.
Methods: The bottom of a parallel-plate flow chamber (87.5 μL) was coated with fibrin (60–240 pmoles), incubated with thrombin (80 pmoles), and washed with buffer (3 mL). A thrombin-specific fluorogenic substrate (SN59) was flowed for 30 min through the chamber (92–322 s^{-1}), and the effluent collected dropwise into hirudin (20 nM). The relative activity of fibrin-associated thrombin was assessed by monitoring the intensity of fluorescence in the effluent. Adhered thrombin was subsequently quantitated by stripping the flow chamber with 1% Triton X-100. In some experiments dabigatran (200 nM) or antithrombin (2.6 μM) were included with the SN59 in the flowing solution.
Results: At constant thrombin and a shear rate of 92 sec^{-1}, increasing the amount of fibrin coating the flow chamber from 60 to 240 pmoles resulted in a 50% increase in the maximum fluorescence signal. Under all these conditions, the signal was relatively stable over 30 min, with decreases ranging from 0 to 20% ($n = 8$). Increasing the shear rate from 92 to 322 sec^{-1} resulted in a 60% decrease in fluorescence. Dabigatran completely inhibited thrombin activity after 10 min with ~30% of the thrombin activity recovered 10 min after removing dabigatran from the fluid phase. Antithrombin showed no inhibition of the fibrin-adhered thrombin over 30 min.
Conclusions: Under flow, the thrombin-fibrin complex displayed unexpected stability and a difference in sensitivity to dabigatran and antithrombin inhibition. This flow model appears suitable for more detailed studies examining the relationship between fibrin structure and clot-bound thrombin stability and function.

BR05

Hemorheologic bases for thrombosis in some myeloproliferative neoplasms

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Background: In today's thrombosis prevention we forget that hemorheologic abnormalities are one from topics of Virchow's triad. Isn't this one of the reasons that venous thromboembolism (VTE) including catheter-related thrombosis (CRT) retain their high frequency despite of modern antithrombotic therapy?
Aims: To study the blood rheological behavior in patients with some myeloproliferative neoplasms.
Methods: Whole blood viscosity (WBV), erythrocytes aggregability, erythrocytes deformability and shear flow were assayed in 16 adults with polycythemia vera (PV), and in 42 young with acute lymphoblastic leukemia (ALL), and in 67 healthy volunteers as the control group. Of patients 38% had thrombosis. Hematological parameters, erythrocyte indices, fibrinogen and B-type natriuretic peptide (BNP) were analyzed too.
Results: Increased WBV revealed totally in PV patients but not for all conditions in ALL patients. WBV was dependent on leukocytes count, on MCH and mainly on MCV. In all patients blood flow properties

different from normal. Both myeloproliferative neoplasms increased totally erythrocyte aggregation but did not violate deformability of red blood cells. Forty percent of patients had elevated BNP assuming subclinical cardiac dysfunction. The latter explains dis-coordinated changes in shear stress values.
(Tau 9.84 mPa in PV, 16.7 mPa in ALL vs 12.2 mPa in donors) required for fully reversible erythrocyte aggregation. As a result, the residual cells units like "erythrocyte-erythrocyte" and/or "erythrocyte-leukocyte" interferes with the laminar flow due to forming of stagnation zones and violates mechanically the blood flow in small vessels.
Conclusions: We found that patients with some myeloproliferative neoplasms have non-hemocoagulation conditions for VTE. The revealed hemorheologic disturbances could be a trigger to start of VTE or to growth of blood clot. Therefore targeted hemorheologic therapy looks attractive in addition to usual VTE prevention.

BR06

Thrombus structure change under various shear rate, using a novel microfluidic device

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Background: Venous thromboembolism (VTE) is one of major causes of sudden death. Prevention: is hard for lacking understanding of mechanical and physical property of venous thrombus.
Aims: Our aim is to extract physical property of venous thrombus to predict the breakdown of venous thrombus preemptively, using multi-scale mechanics simulation model and mathematical model of biological structure.
Methods: To meet our aim, we made a microfluidic device system to observe thrombus structure formed under various but venous range shear rate conditions as a basic experimental technology for mathematical analysis. The inside of the channel was fully siliconized and partly coated with collagen. Fluorescently-stained blood was flowed with venous shear rate by a home build pressure control pumping device. The thrombus imaged was observed by a fluorescence microscope.
Results: The structures of thrombus under various shear rate were much different from each other even in normal venous shear rate range.
1. Fibrin fiber orientation was random at 0 s-1 shear rate. As increasing shear rate, ratio of fibrin fiber aligned along the direction of flow was increased.
2. As increasing shear rate, thickness of fibrin fibers were increased.
3. As increasing shear rate, mean length of fibrin fiber was increased.
4. As increasing shear rate, fluorescent intensity of the fibrin fiber was increased.
Conclusions: Basically our microfluidics system was useful to observe the thrombus structure formed under various conditions. The structure is essential for the mechanical and physical property of venous thrombus. This property should be important for the understanding VTE because VTE is caused by breakdown of the venous thrombus.
We made a novel microfluidic system to visualize the venous thrombus structure. The shear rate affect thrombus structures even in normal venous shear range. Our results can give much information for mathematical modeling of VTE and will be beneficial for understanding VTE.

Control of Anticoagulation

CA01

Effect of extremes of body weight on efficacy and safety of rivaroxaban in the treatment of venous thromboembolism; real life experience

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Background: Although routine monitoring of rivaroxaban is not required, assessment of drug levels may be useful in some situations including "extremes in body weight" [EBW] (< 50 kg or >120 kg).
Aims: To assess the effect of EBW on efficacy and safety of rivaroxaban in the treatment of venous thromboembolism (VTE).
Methods: An unselected group of 219 patients with VTE (53% deep vein thrombosis [DVT], 41% pulmonary embolism [PE] and 6% PE and DVT: 51% male, 49% female, mean age 59 years) treated with rivaroxaban had drug levels measured for various reasons over 15 months. Bleeding episodes and recurrent VTE were recorded over a follow-up of 6–18 months as a service evaluation.
Results: Patients were divided into three groups based on weight (< 50 kg: n = 20, 50–120 kg: n = 135 and >120 kg: n = 45). Weights were unavailable in 19/219 and were excluded. Only samples taken 2–4 h after rivaroxaban 20 mg (167/200) were compared (< 50 kg: n = 18, 45% PE, 50–120 kg: n = 105, 38% PE and >120 kg: n = 44, 40% PE). Mean weight [range] (kg) in the 3 groups were 43 [38–49], 86 [50–120)] and 135 [121–186]. All patients had ALT < 1.5 of upper normal limit and creatinine clearance >30 mL/min. Patients with weight < 50 kg had significantly higher rivaroxaban levels (median (ng/mL) [95% confidence interval]) 460 [380–601] compared to patients with weight 50–120 kg: 308 [308–381] and >120 kg: 281[242–327], (P = 0.005). Furthermore, 3/18 (16.7%) patients with weight < 50 kg had rivaroxaban levels >700 ng/mL. Although they did not have significant bleeding, rivaroxaban dose was reduced to 15 mg daily. Overall clinically relevant, major bleeding and recurrent VTE rates were 9.5%, 1% and 3.1% respectively, however these were not associated with rivaroxaban levels or patient weight.
Conclusions: Our study demonstrates the wide range of rivaroxaban levels obtained at EBW. In particular the levels in low weight patients are significantly elevated. Although, these were not correlated with bleeding, larger studies are required to establish safety in this group.

CA02

VTE Prevention in Acutely Ill Medical Patients with Extended Duration Betrixaban – A Multicenter, Randomized, Active-Controlled Efficacy and Safety Study Comparing Extended Duration Betrixaban with Standard of Care Enoxaparin

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Background: The majority of venous thromboembolism (VTE) events among hospitalized acutely ill medical patients occur following hospital discharge. Betrixaban is a novel oral factor Xa inhibitor with a half-life of 18-27 hours. Approximately 7% of the administered dose is excreted renally.
Methods: APEX is a phase III multicenter, randomized, double blind, active-controlled trial that assesses the safety and efficacy of extended prophylaxis with oral betrixaban (35 + 7 days) compared with standard of care injections of enoxaparin (10 ± 4 days) for preventing VTE among acute medically ill patients. The primary efficacy endpoint was the composite of symptomatic proximal or distal deep vein thrombosis (DVT), non-fatal pulmonary embolism (PE), VTE-related death, or asymptomatic proximal DVT through 35 + 7 days. The primary safety endpoint was the occurrence of ISTH major bleeding events through 7 days after drug discontinuation.
Results: A total of 7,513 patients were randomized. Betrixaban was associated with a reduction in the primary efficacy endpoint in the first primary analysis cohort of patients with a D-Dimer > 2X ULN measured by local assay (RRR = 19.4%; p = 0.054). Betrixaban reduced the primary efficacy endpoint in the overall efficacy population (RRR = 24.0%; p = 0.006). Betrixaban was not associated with a significant increase in major or fatal bleeding.
Conclusion: In acutely ill medical patients, extended duration betrixaban thromboprophylaxis reduced venous thromboembolic events in the overall study population when compared with standard of care enoxaparin.

CA03

Improving clinical management of VTE: simulation in continuing education

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Background: Evidence-based anticoagulant therapy is often not implemented in patients with venous thromboembolism (VTE).
Aims: To determine if an online, simulation-based continuing medical education (CME) intervention could improve performance of hematologists/oncologists and cardiologists in managing patients with VTE.
Methods: The intervention consisted of two patient case simulations presented in an advanced, interactive platform. The format allowed learners to make decisions regarding lab tests, diagnoses and treatments. Importantly, these decisions were not limited by multiple choice; rather the platform's interface allowed any decision possible in the scope and depth of actual practice The clinical decisions made by the participants were analyzed using an artificial intelligence engine, and clinical guidance was provided based on current evidence. Participant decisions were collected after clinical guidance and compared with baseline data using a 2-tailed paired T-test to provide *P* values and assess the impact of education on clinical decisions.
Results: The assessment sample consisted of 146 hematologists/oncologists and 216 cardiologists who made clinical decisions. As a result of clinical guidance, significant relative improvements were observed:

- 65% improvement in orders for the sPESI score for hematologists/oncologists (54% vs 33%; $P < 0.001$) and a 60% improvement for cardiologists (58% vs 36%; $P < 0.001$).
- 36% improvement in appropriate diagnosis of PE for hematologists/oncologists (68% vs 56%; $P < 0.001$) and a 36% improvement for cardiologists (71% vs 52%; $P < 0.001$).
- 27% improvement in orders to withhold rivaroxaban 24 h prior to elective surgery for hematologists/oncologists (85% vs 67%; $P = 0.001$) and a 36% improvement for cardiologists (76% vs 56%: $P < 0.001$).

Conclusions: This study demonstrates that simulation-based CME can improve evidence-based practice decisions of specialists, and suggests that this type of intervention can improve patient outcomes.

CA04

Genetic dosing algorithm underestimates the warfarin dose requirement among patients having severe thrombosis or thrombophilia

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Background: Warfarin metabolism and the required effective dose vary significantly among individuals. Genetic variability in *CYP2C9* and *VKORC1* together with patient age and size have been shown to explain 50–60% of interindividual variability in warfarin dose requirement.
Aims: We examined the accuracy of genetic estimation of warfarin dosing among patients with severe thrombosis and/or thrombophilia by comparing with the clinically established INR-based dose.
Methods: Study group consisted of 47 patients with severe thrombosis or thrombophilia, referred to the Comprehensive Coagulation Center for consultation. Patients were screened for common thrombophilias and genotyped for *CYP2C9*2*, *CYP2C9*3* and *VKORC1*-1639A and -1173T variants. Warfarin dose requirement was estimated using a pharmacogenetic dosing algorithm. Subsequently, at the clinic, the administered warfarin dose was tailored, supplementing the genotype with comprehensive clinical, warfarin dosing algorithms, and INR data.
Results: Mean patient age was 45 years (17–76), height 167 cm (144–184) and weight 76 kg (50–166), 57% being women. Twenty-nine (61%) had previous venous or arterial thrombosis and 28 (60%) had a severe genetic or acquired thrombophilia. Pharmacogenetic dosing algorithm estimated warfarin dose requirement to be on average 5.1 mg/day (SD 1.7 mg/day, range 1.4 to 8.4 mg/day), whereas the actual required dose was significantly higher, 7.3 mg/day (SD 5.1 mg/day, range 0.8 to 30.0 mg/day, $P < 0.005$) after clinical visit and assessment of the other medications, gender, BMI, and reaching and maintaining therapeutic INR.
Conclusions: Genetic profiling may aid with the dosing of warfarin. However, in patients with severe thrombosis and/or thrombophilia, many other factors impact the appropriate dose, among others enhanced thrombin generation. Pharmacogenetic dosing algorithms, based mainly on patients with atrial fibrillation, may significantly underestimate warfarin dose requirement in these patients.

CA05

Impact of apixaban on the Dilute Russell's viper venom time: an ex-vivo study

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Background: The dilute Russell's Viper Venom Time (dRVVT) has been proposed for screening the intensity of anticoagulation of all

direct oral anticoagulants. In patient plasma samples, dRVVT correlated well with dabigatran and rivaroxaban concentrations. However, less is known about the ex-vivo influence of apixaban on dRVVT.
Aims: This study aimed at comparing dRVVT measurements with estimated plasma concentrations of apixaban in clinical samples.
Methods: Fifty-four plasma samples from patients treated with apixaban were included in the study. The dRVVT was performed prospectively. A screening (STA®-Staclot®DRVV-Screen) and a confirmatory testing (STA®-Staclot®DRVV-Confirm) were used, containing low and high phospholipid concentrations respectively. Apixaban plasma concentrations were estimated prospectively with the Biophen® Direct FXa Inhibitor, a specific chromogenic anti-Xa assay.
Results: Apixaban plasma concentrations ranged from 0 to 395 ng/ml. The dRVVT yielded a curvilinear concentration-dependent prolongation of clotting time (Figure 1).

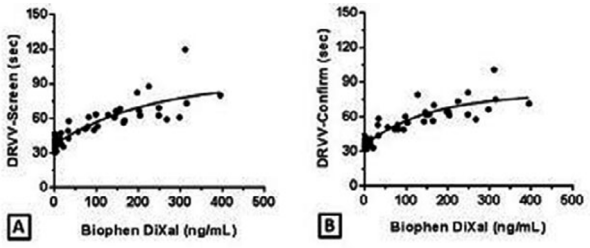

Figure 1: correlation between dRVVT and estimated plasma concentrations of apixaban (A: DRVV-Screen, B: DRVV-Confirm)

The Spearman correlation coefficient between dRVVT and estimated plasma concentration of apixaban was 0.90 (95% CI: 0.83 to 0.94; $P < 0.0001$; r^2 for non-linear regression = 0.75) and 0.89 (95% CI: 0.82 to 0.94; $P < 0.0001$; r^2 for non-linear regression = 0.82) for DRVV-Screen and DRVV-Confirm respectively. ROC curves gave a cut-off to exclude *supra*-therapeutic levels at C_{trough} (i.e. 230 ng/ml) of 58.4 sec for DRVV-Screen and 56.6 sec for DRVV-Confirm (sensitivity 100% (95% CI, 100.0%-100.0%), negative predictive value 100%).
Conclusions: The dRVVT correlated well with estimated plasma concentrations of apixaban. DRVV-Screen and DRVV-Confirm showed the same sensitivity. DRVV-Confirm should nevertheless be preferred since this assay is expected to be less affected by possible lupus anticoagulants. These results support the use of the dRVVT as a simple and rapid-to-perform assay to exclude supra-therapeutic concentrations of all direct oral anticoagulants.

CA06

Choosing wisely: the impact of inclusion and exclusion criteria on efficacy and safety outcomes in EINSTEINDVT/PE and AMPLIFY

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Background: Several direct oral anticoagulants (DOAC) have been tested against standard of care (SOC) in the treatment of venous thromboembolism (VTE). The interpretation of indirect comparisons and meta-analyses of DOAC VTE trials can be influenced by differences in the phase-III trials included.
Aims: To evaluate the effects of trial design and exclusion criteria on results, we calculated outcome events in EINSTEIN DVT/PE separately for patients who would or would not have met the eligibility criteria of AMPLIFY.
Methods: Treatment duration of EINSTEIN DVT/PE trial patients was adjusted to the AMPLIFY design by excluding patients with an intended treatment duration of 3 months and by truncating all other patients at 6 months. Next, exclusion criteria of AMPLIFY were applied to separate an EINSTEIN DVT/PE cohort that mimicked the patient population of AMPLIFY (cohort 1) from a cohort that would not have been eligible for AMPLIFY (cohort 2). For these cohorts, separate outcome analyses were performed.
Results: Cohort 1 consisted of 5263 patients who mimicked the patient population of AMPLIFY and cohort 2 included 2358 patients who did not. Patients in cohort 1 were older, more often male, more often had unprovoked VTE, a previous VTE or known thrombophilia than patients in cohort 2.

Table 1. Patient characteristics and clinical outcomes of EINSTEIN DVT/PE patients who were and were not potentially eligible for the AMPLIFY study.

	Cohort 1: EINSTEIN DVT/PE patients **eligible** for AMPLIFY N=5263	Cohort 2: EINSTEIN DVT/PE patients **ineligible** for AMPLIFY N=2358	P-value eligible vs ineligible	AMPLIFY population N=5395
Age — yr	59.4±16.0	52.1±17.6	P<0.0001	57.6±16.0
Male sex — no. (%)	3129 (59.5)	1053 (44.7)	P<0.0001	3167 (58.7)
Weight — Mean — kg	83.51±18.4	81.79±19.5	P=0.0002	84.6±19.6
Weight Distribution — no.				
≤60 kg	469 (8.9)	302 (12.8)	P<0.0001	476(8.8)
>60 to < 100 kg	3963 (75.8)	1730 (73.5)		3868 (71.7)
> 100 kg	805 (15.3)	322 (13.7)		1040 (19.3)
Data missing	6 (0.1)	4 (0.2)		11 (0.2)
Qualifying diagnosis — no. (%)			P=0.037	
DVT	2102 (39.9)	884 (37.5)		3532 (65.5)
PE	2286 (43.4)	1107 (46.9)		1359 (25.2)
PE with DVT	822 (15.6)	348 (14.8)		477 (8.8)
Could not be evaluated	53 (1.0)	19 (0.8)		27 (0.5)
Time from onset of symptoms to randomization — days			P<0.0001	
Median	5.0	4.0		5.0
Interquartile range	(2.0-10.0)	(2.0-8.0)		(3.0-9.0)
Clinical presentation of VTE — no. (%)			P<0.0001	
Unprovoked	4806 (91.3)	253 (10.7)		4845 (89.8)
Provoked	457 (8.7)	2105 (89.3)		544 (10.1)
Not reported	-	-		6 (0.1)
Risk factors for recurrent VTE — no. (%)‡				
Previous VTE	1352 (25.7)	227 (9.6)	P<0.0001	872 (16.2)
Known thrombophilia	433 (8.2)	41 (1.7)	P<0.0001	133 (2.4)
Active cancer	292 (5.5)	110 (4.7)		143 (2.7)
Treatment with LMWH, heparin, or fondaparinux before randomization — no. (%)			P=0.10	
None	767 (14.6)	372 (15.8)		739 (13.7)
≤12 hr	461 (8.8)	213 (9.0)		712 (13.2)
>12 to 24 hr	2702 (51.3)	1130 (47.9)		2242 (41.6)
>24 to 48 hr	1266 (24.1)	610 (25.9)		1642 (30.4)
>48 hr	67 (1.3)	33 (1.4)		48 (0.9)
Data missing	-	-		12 (0.2)
Recurrent VTE	28 (1.5%) vs 61 (2.3%)	31 (2.6%) vs 27 (2.3%)		59 (2.3%) vs 71 (2.7%)
NOAC vs LMWH/VKA	**RR 0.63 (0.42 to 0.94; p=0.026)**	**RR 1.08 (0.65 to 1.78; p=0.76)**		**RR 0.84 (0.60-1.18)**
Major bleeding	22 (0.9%) vs 46 (1.7%)	14 (1.3%) vs 12 (1.1%)		53 (1.0%) vs 49 (1.8%)
NOAC vs LMWH/VKA	**RR 0.49 (0.29 to 0.80; p=0.0049)**	**RR 1.13 (0.52 to 2.43; p=0.76)**		**RR 0.31 (0.17-0.55)**

Rivaroxaban significantly reduced recurrent VTE compared to SOC in cohort 1 but not in cohort 2. Major bleeding was significantly reduced by rivaroxaban compared to SOC in cohort 1 but not in cohort 2.
Conclusions: EINSTEIN patients excluded by AMPLIFY eligibility criteria had an almost 1.5 to 2 times increased risk of major bleeding and recurrent venous thromboembolic complications compared to those who fitted the AMPLIFY eligibility criteria. The results of this analysis highlight the difficulties in comparing data from different studies with non-comparable populations. To increase external validity, clinical trials should apply limited exclusion criteria.

CA07

Optimizing therapy for vitamin k antagonist induced major hemorrhage: a systematic review

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Background: Vitamin K Antagonists (VKA) remains the main stay of oral anticoagulant therapy for prevention and treatment of thromboembolism. Warfarin induced bleeding is associated with significant morbidity and mortality.
Aims: This review is aimed at examining the current best evidence regarding optimal therapy for VKA induced major bleeding.
Methods: The PUBMED, MEDLINE and EMBASE were searched for relevant English-language reports of clinical trials and studies, for the current best evidence regarding the optimal dosing, efficacy and

safety of the different warfarin reversal agents used in the setting of major bleeding.

Results: Our search yielded 1400 eligible studies.There were variations in individual studies with respect to study size, treatment protocols, Prothrombin Complex Concentrate (PCC) dosing, and additional interventions. Only one high-quality RCT found PCC to be non-inferior to plasma with respect to hemostatic efficacy, mortality, and adverse events ($P < 0.05$). In the remainder of the studies, efficacy was assessed mainly by INR correction, and the frequency of patients that achieved INR correction was significantly greater in the PCC group compared to plasma group. Reversal of warfarin anticoagulation for major bleeding demands administration of vitamin K to re-establish the activity of vitamin K-dependent coagulation factors, an effect seen at least 6 h after intravenous administration. Adjunctive replacement of coagulation factors with PCC or plasma contribute to the rapid normalization of hemostasis desired.

Conclusions: There is moderate quality evidence that supports using PCCs or plasma as a supplement to vitamin K for urgent reversal of VKA-associated hemorrhage. Current reports does not clearly indicate if the outcome at 24 h would have been different after vitamin K and FFP infusion or after vitamin K alone. Considering the morbidity and mortality associated with warfarin-related hemorrhage, the need for high-quality studies evaluating patients clinical outcomes is hereby emphasized.

CA08

The effect of andexanet alfa on the pharmacokinetics and renal clearance of the direct factor Xa inhibitors apixaban, rivaroxaban, edodoxaban and betrixaban

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Background: Andexanet alfa (AnXa) is being developed to reverse anticoagulation due to fXa inhibitors in case of major bleeding or if urgent surgery is required. AnXa is a decoy derivative of fXa that sequesters fXa inhibitors and has neither pro- nor anti-coagulant activity on its own. AnXa binding to direct fXa inhibitors affects their pharmacokinetics (PK). Following AnXa administration fXa inhibitors redistribute to the vasculature, increasing the total concentration several fold while unbound concentrations and fXa inhibition decrease.

Aims: To determine if AnXa alters the CL mechanisms of the direct fXa inhibitors: apixaban, rivaroxaban, edoxaban, and betrixaban.

Methods: Double-blind placebo controlled studies evaluated the ability of AnXa to reverse anticoagulation for each direct fXa inhibitor. Total apparent and renal clearance values (CLss/F and Clr) for the fXa inhibitors were compared in the presence and absence of AnXa to determine if AnXa altered the proportion of CLr in healthy subjects with normal renal function. PK data the day before and on the day of AnXa dosing were compared. Urine samples were collected on both days at 0–6, 6–12, 12–24 hrs post fXa inhibitor dose. HPLC-MS/MS was used to quantify fXa inhibitors. Standard PK parameters for all subjects were calculated using Phoenix WinNonLin (v. 6.4): C_{max}, $AUC_{0\text{-}tau}$, $AUC_{0\text{-}inf}$, CLss/F, and CLr.

Results: Increasing AnXa doses decreased CLss/F without altering % CLr. AnXa administration decreased CLss/F by up to 6-fold (Table 1).

Table 1 Shows the mean ± SD values for Clss/F, Clr and % CLr for each fXa inhibitor on the day prior to and the day of AnXa administration at the highest AnXa dose used for each fXa inhibitor.

FXa Inhibitor	Day	AnXa Dose Regimen	CLss/F (L/hr)	CLr (L/hr)	%CLr
Apixaban	5	Untreated	5.0 ± 0.54	1.2 ± 0.22	23.8 ± 4.4
	6	420 mg bolus + 4 mg/min over 2 hr	2.3 ± 0.30	0.57 ± 0.11	25.0 ± 2.9
Rivaroxaban	5	Untreated	8.2 ± 1.1	3.1 ± 0.51	37.5 ± 4.8
	6	800 mg bolus + 8 mg/min over 2 hr	2.9 ± 0.55	1.1 ± 0.17	38.4 ± 8.4
Edoxaban	5	Untreated	39.2 ± 6.2	10.8 ± 2.8	28.2 ± 7.9
	6	800 mg bolus + 8 mg/min over 1 hr	12.6 ± 2.8	4.0 ± 1.0	32.3 ± 7.2
Betrixaban	6	Untreated	221 ± 68	6.0 ± 2.3	2.84 ± 1.33
	7	800 mg bolus ± 8 mg/min over 2 hr	34 ± 14	2.2 ± 1.0	6.68 ± 2.48

[fXa Inhibitor %CLr +/- AnXa]

Conclusions: Tight binding of AnXa to fXa inhibitors decreased unbound levels, reducing what was available for elimination or metabolism. This, in turn, decreased CLss/F. However, the relative proportion of CLr to CLss/F remained consistent for each direct fXa inhibitor, indicating that AnXa does not alter clearance mechanisms.

CA09

Prothrombin conversion and thrombin inactivation in patients anticoagulated with enoxaparin, vitamin K antagonists, and rivaroxaban

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Background: The thrombin generation (TG) test assesses the hemostatic capacity of an individual and is indicative of the bleeding and thrombosis risk. TG depends on the balance of the pro- and anticoagulant processes in clotting plasma, which can be measured with the thrombin dynamics method.

Aims: Anticoagulant therapy is known to cause large inter-individual variation in TG. In this study we aim to pinpoint the anticoagulant effect of enoxaparin, vitamin K antagonists and rivaroxaban on TG to the pro- or anticoagulant pathway.

Methods: TG was measured in 202 healthy subjects, 30 patients on enoxaparin, 129 on vitamin-K antagonists (VKA), and 12 on rivaroxaban. Thrombin dynamics were determined through the computational extraction of prothrombin conversion and thrombin inactivation from TG curves.

Results: All anticoagulants reduce the ETP and peak height significantly and VKA and rivaroxaban prolong the lagtime and time-to-peak (Figure 1). On the procoagulant side, VKA and rivaroxaban treatment diminished the total amount of prothrombin converted during TG (PC_{tot}, Figure 2). Rivaroxaban attenuated the maximum activity of the prothrombinase complex (PC_{max}) the most (-95%, $P < 0.001$), followed by VKA (-68%, $P < 0.001$). Enoxaparin did not have a significant effect on prothrombin conversion. Enoxaparin increased the thrombin decay capacity (TDC) (+21%, $P = 0.009$), whereas VKAs and rivaroxaban slightly reduce TDC, and thrombin-antithrombin formation is reduced in VKA and rivaroxaban samples.

Conclusions: Rivaroxaban reduces TG mainly by reducing PC_{max}, causing a subsequent lowering of PC_{tot}. VKAs reduce both PC_{tot} and PC_{max}, whereas enoxaparin decreases TG through the stimulation of

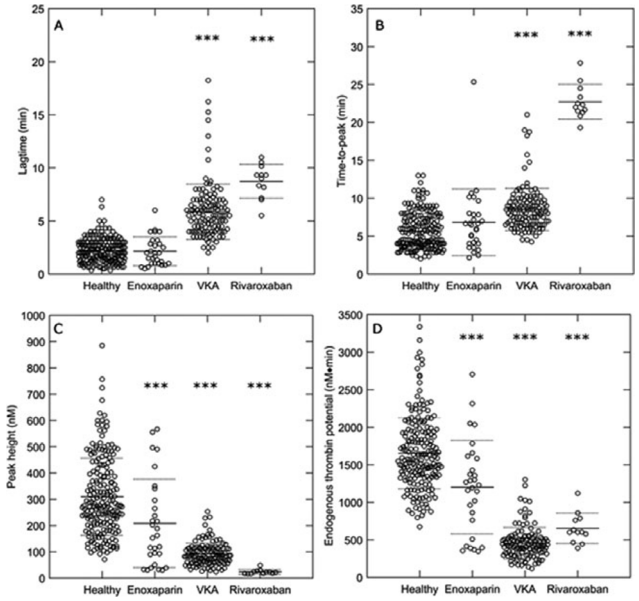

Figure 1 Thrombin generation. (Abstract CA09)

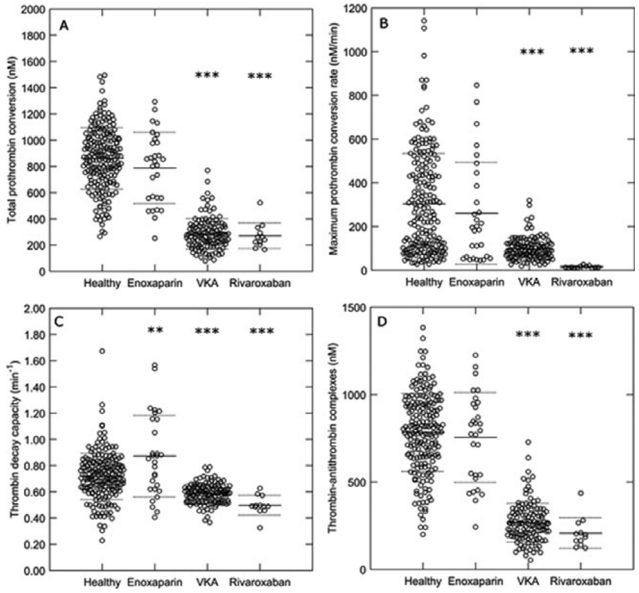

Figure 2 Thrombin dynamics. (Abstract CA09)

thrombin inactivation (TDC) and not via the inhibition of prothrombin conversion.

CA10

Individual variability of plasma with edoxaban on the fibrin structure as a function of blood donors

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Background: The thickness of the fibrin clot fibers plays an important role on its stability and resistance to fibrinolysis. The fibrin nanostructure profile has been proposed as a tool for determining the hemorrhagic and thrombotic risk, especially with DOA [1], for which very few data are available in the literature.

Aims: Determine the individual variability of plasma from human blood donors spiked with edoxaban on the fibrin structure.

Methods: Plasmas from 132 blood donors were collected from 66 men and 66 women and selected on a normal fibrinogen concentration. These plasmas were randomly assigned into 2 groups to be spiked with edoxaban at 50 or 150 ng/mL. Each plasma was spiked with DMSO solvent used as control.

All plasmas were incubated with a low TF concentration and t-PA, then the coagulation was triggered by calcium. The number of protofibrils (Np) and the fibrin nanostructure parameters were determined on a STA® prototype [2]. The results were normalized with each control plasma and the individual fibrin nanostructure profiles were compared during coagulation and fibrinolysis.

Results: With edoxaban concentration, all the temporal parameters were lengthened during fibrin formation. Np also increased and since the clot stability decreased due to a looser fiber structure less resistant to lysis, especially in women and 50 ng/mL edoxaban. The lysis rate increased and the lysis to fibrin formation ratio decreased regardless of gender.

Conclusions: Edoxaban modified the fibrin structure as a function of the blood donors, making the fibrin clot less resistant to lysis. The coagulo-lytic balance bends to a better susceptibility to lysis whatever the gender. The fibrin structure could be a useful tool in clinical practice for the individual management of bleeding disorders.

[1] Dassi et al. ISTH 15 ABS-3535;
[2] Dassi et al., ISTH 15 ABS-3593

CA11

Accuracy of the louzada prediction rule for recurrent VTE in Cancer patients on rivaroxaban for the treatment of venous thromboembolic disease

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Background: We previously demonstrated that a prediction tool for recurrence may stratify VTE recurrence risk in cancer patients but it was derived in patients treated with LMWH.

Aims: To determine the accuracy of two systems of scoring for prediction of recurrent VTE in cancer patients on rivaroxaban.

Methods: retrospective chart review in cancer patients treated with rivaroxaban, categorizing patients by 2 systems: The first (call it scoring system A) assigned a score of -2 for those with TMN stage I cancer and the second (system B) assigned a score of -1 for either stage I or II, in additions to the other predictors.

Results: 237 patients received rivaroxaban; 65 were initiated, 3 recurred, 75 started between day 8 and the 6 month point, 3 also recurred and 97 started at 6 months or beyond with again 3 recurrences. The average duration of rivaroxaban therapy was 297 days; Analyzing patients only during their time on rivaroxaban, and combining all time periods, 3 patients of 143 (2.0%) recurred if system A scored ≤ 0 and 3 of 153 (2.0%) if system B scored ≤ 0. There were 6 patients of 94 (6.4%) who recurred if system A scored > 0 and 6 of 84 (7.1%) if system B scored > 0. Including all patients and all treatment times regardless of whether they were on LMWH or rivaroxaban 19 of 143 (13.3%) recurred if system A scored ≤ 0 and 20 of 153 (13.1%) if system B scored ≤ 0, and 21 patients of 94 (22.3%) who recurred if system A scored > 0 and 20 of 84 (23.8%) if system B scored > 0.

Conclusions: Our study suggests the recurrence prediction tool does accurately predict two different recurrence risk groups and that system A and B give similar results. The recurrence risk is acceptable on rivaroxaban, and rivaroxaban can be considered acceptable therapy

for the treatment of cancer associated with VTE, both at the time of initial diagnoses and after therapy with LMWH.

CA12

Early changes in medication beliefs, illness perceptions and anticoagulation related quality of life in patients poorly controlled on vitamin-K antagonists (VKAs) switched to a direct oral anticoagulant (DOAC) - results from the SWITCHING study

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Background: The SWITCHING study has previously reported that those poorly controlled on VKAs (TTR< 50%) have distinct illness perceptions, medication beliefs and worse quality of life (QoL) compared to those who are well controlled (TTR>75%). Little research exists describing changes in these factors and their impact on adherence for those with poor control on VKAs switched to a DOAC as per guidelines.

Aims: To describe early changes in medication beliefs, illness perceptions and anticoagulant related QoL in a cohort of patients poorly controlled on VKAs (TTR < 50%) and how they change, if at all, 1 month post switching therapy to a DOAC.

Methods: Eligible patients were recruited from the anticoagulation clinics at King's College Hospital, and following informed consent, completed a questionnaire pack comprising the following instruments: revised illness perceptions questionnaire (IPQ-R), beliefs about medicines questionnaire (BMQ) and anti-clot treatment scale (ACTS) whilst on warfarin (T0) and again 1 month post switch (T1). Differences between time points were compared using the Wilcoxon rank test with significance considered at $P < 0.05$. The study was approved by the London-Dulwich ethics committee.

Results: At the time of analysis, 142 patients had switched, 63% male, 77% AF and 19% VTE. Mean age was 68 years (SD = 13), mean duration on VKA was 187 weeks (SD=191), mean HAS-BLED 2.3 (SD=0.8), with the AF sub-cohort having a mean CHA_2DS_2VASc of 3.3 (SD=1.5). Apixaban was prescribed for 56% of patients and rivaroxaban for the remainder. Table 1 outlines the significant changes observed post switch.

Conclusions: Our results demonstrate that patients experience significant early changes in beliefs and QoL following a switch to a DOAC, representing a shift towards belief patterns predictive of improved adherence. It is important to establish if these improvements are maintained long-term and how changes can be harnessed to improve long-term adherence to anticoagulation medication.

Table 1 Summary of significant changes in beliefs. (Abstract CA12)

Questionnaire	Subscale and sample questions	AF Only (n = 109) Z Score (T1–T0)	p	VTE Only (n = 27) Z Score (T1–T0)	p	All Indications (n = 142) Z Score (T1–T0)	p
IPQ-R: Illness Perceptions	Illness Coherence (Patient self-reported understanding of illness e.g. 'I have a clear picture or understanding of my condition')	-0.488	0.626	2.475	**0.013**	0.565	0.572
	Emotional Representation (Emotional response evoked by illness e.g. 'When I think about my condition I get upset)	-2.129	**0.033**	0.488	0.625	-1.648	0.099
BMQ: Medication Beliefs	General Overuse (Belief that in general medicines are overused in healthcare e.g. 'Doctors place too much trust on medicines')	-1.981	**0.048**	0.293	0.770	-1.998	**0.046**
	Specific Necessity (Patient's perceived need for anticoagulation therapy e.g. 'My health at present depends on VKA/DOAC')	-1.155	0.248	0.123	0.902	-1.278	0.201
	Specific Concerns (Patient anticoagulation specific concerns e.g. 'Having to take VKA/DOAC worries me')	-5.089	**< 0.001**	-1.768	0.077	-5.461	**< 0.001**
ACTS: Anticoagulation Specific QoL	Burdens (Anticoagulation specific impediments to quality of life e.g. 'Overall, how much of a negative impact has VKA/DOAC had on your life?')	-7.661	**< 0.001**	-3.250	**0.001**	-8.505	**< 0.001**
	Benefits (Patient reported gains from anticoagulation therapy e.g. 'Overall, how much of a positive impact has VKA/DOAC treatment had on your life?')	2.934	**0.003**	-1.049	0.294	2.169	**0.030**

Responses are on a Likert scale ranging from 'strongly agree' to 'strongly disagree' for IPQ-R and BMQ, and 'not at all' to 'extremely' for ACTS. Subscales with no significant changes include: IPQ-R; Timeline Acute/Chronic, Timeline Cyclical, Consequences, Personal Control, Treatment Control and BMQ; General Harm. Z score indicates magnitude of change, with positive signs indicating an increase in subscale score and negative sign indicating a decrease in subscale score

CA13

Analysis of heparin induced thrombocytopenia screening tests in critical care units

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Background: Heparin induced thrombocytopenia (HIT) is a consequence of atypical immunological response resulting in a significant drop in platelet count. Instead of bleeding, this phenomenon leads frequently and paradoxically to thrombotic event by platelet activation. It is estimated 1 in 5000 hospitalized patients and 1 in 0.1–0.5% individuals exposed to heparin developed HIT. Recent publications have shown inconsistency in increased HIT incidence among critically ill individuals.
Aims: This quality improvement study estimated the HIT burden in our intensive care unit (ICU) in relation to whole institute and international figures.
Methods: A retrospective observational study reviewed 1,238 HIT screening tests for patients receiving UH and/or LMWH with clinical suspicion of HIT by two screening methods from 2011 to 2014. The diamed rapid particle gel Immunoassay (Di-PaGIA) and enzyme linked immunosorbent assay (ELISA) screening methods were used to identify and semi-quantify Heparin/PF4 antibody complex.
Results: There were 340 tested samples from patients in ICU with age ranging from 1 week to 111 years (median, 62 years). Out of those samples, 12.94% were positive by one or both used methods. In this cohort, 232 patients were exposed to unfractionated heparin (UH), 31 to low molecular weight heparin (LMWH) and 77 to both medications. The tests reported positive for HIT 16.81% in patients exposed to UH and 6.49% for patients who received both medications and none tested positive for those who received LMWH alone. The reported prevalence of HIT positivity in all requested tests (1,238) was 7.35%.
Conclusions: These results are in concordance with what has been reported as an increase incidence of HIT among critically ill patients. UH clearly is more associated with developing HIT when compared with LMWH administration. This could be used as a surrogate indicator for quality assurance in HIT awareness among ICU physicians. Further large scale prospective study is needed to confirm these observations.

CA14

Post analytical external quality assessment (EQA) for computerised decision support software (CDSS) employed for INR dosing

Kitchen DP, Munroe-Peart S, Brown L, Jennings I, Kitchen S, Woods TAL and Walker ID

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Background: UK NEQAS for Blood Coagulation provides EQA for INR testing in both laboratory and Point of Care (POC) setting for many years. However an EQA programme for monitoring the dosing of vitamin K antagonist (VKA) following an INR test does not exist.
Aims: To introduce of an EQA programme for assessment of the VKA dosing.
Methods: A pilot study was performed with a virtual patient scenario of INR results and dates together with a short clinical history. Users provide a dosage and recall time for the patient using their routine method. Users that were dosing manually could also take part. The survey was open to both the laboratory and POC based users in UK NEQAS BC INR programmes.
Results: 324 centres returned results with 26% from laboratory programme and 61% from the POC programme (the remainder entering data anonymously). The virtual patient's results were INR of 2.7, 2.9, 3.4, 3.8 on day 35, day 14, day 7 and day 0 respectively on a constant dose of 7 mg per day of warfarin. Dosage recommendations ranged from 0 to 8 mg per day. 90% of centres suggested a dose of between 6 and 7 mg per day with a further 8% recommending a dose of between 5 and < 6 mg per day. The highest dose of 8 mg/day was stated by only one centre. Recall intervals ranged from 2 days to 28 days with a median of 7 days (n = 214 centres). 39% of dosing for this virtual patient was performed manually and 39% was performed using the CDSS recommendation (13 different systems). The remaining 22% used the CDSS but varied either the dose (8%) or the recall period (4%) or both (10%). Nurses were most often performing the exercise (49%) with doctors (19%), health care assistants (15%), laboratory scientists (15%), pharmacists (6%) and a practice manager also taking part.
Conclusions: Good agreement was seen in this first exercise with 90% recommending a dose of between 6 and 7 mg per day and 78% giving a recall time of 7 days. However we feel that post analytical EQA is required to bring standardisation to this area and highlight differences to users.

CA15

external quality assurance provision (EQA) for the point of care Xprecia stride INR device

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Background: UK NEQAS for Blood Coagulation have been providing EQA for INR testing both in the laboratory and in a Point of Care (POC) setting for many years. The Xprecia Stride POC INR device has been introduced to the market and UK NEQAS BC has assessed EQA for this device.
Aims: To evaluate the provision of EQA for the Xprecia Stride INR device.
Methods: In house studies were used to determine the most appropriate materials required to allow testing to be performed with the Xprecia Stride. The Xprecia Stride requires 6 μL of fresh capillary whole blood to obtain an INR result. It is not possible to provide fresh whole blood as an EQA material but citrated plasma was obtained from genuine patients receiving Vitamin K antagonist therapy as it is important to provide samples that are as near as possible to those for which the device is calibrated. The lyophilised samples are reconstituted using calcium chloride (provided) in order to form a clot when applied to the device. When the most appropriate calcium chloride and plasma mix had been determined a pilot exercise was performed with centres that were using the Xprecia stride. The material was posted to users. 2 samples were provided.
Results: The survey was distributed to 15 users. The median INR value was calculated and a target range (+/-15% deviation from the median result) was calculated in line with existing UK NEQAS BC INR EQA programmes. Between centre variation in results was comparable to that seen with other devices in our EQA programmes.

Table Results of first survey for EQA for Xprecia Stride.

	Sample 1	Sample 2
Number of results	10	10
Median	3.7	4.95
Coefficient of variation (CV)	9.8%	7.9%
Target Range (15% deviation from median)	3.1–4.3	4.2–5.7
Percentage outside Target range	10%	0%

Conclusions: The EQA material was deemed to be suitable to perform EQA checks on the Xprecia stride device and UK NEQAS BC will conduct further pilot studies followed by a full programme for this device.

CA16

An ultrasound-based sensor for real-time monitoring of heparin anticoagulation therapy

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Background: Heparin anticoagulation therapy has a narrow therapeutic window and is the second most common ICU medication error. The aPTT monitors heparin, but suffers from long turnaround times, a variable reference range, limited utility with low molecular weight heparin (LMWH), and poor correlation to outcome. Here, we describe a photoacoustic imaging (light in-thermal expansion-sound out) technique to monitor heparin anticoagulation therapy in real time.
Aims: Aim 1. Measure heparin concentrations *in vitro*.
Aim 2. Measure heparin concentrations in a mouse model of anticoagulation therapy.
Methods: In Aim 1, 10 uL of 50 to 5000 U/mL heparin was added to 100 uL of fresh human blood followed by 100 uL of 1.6 mM methylene blue. Samples were loaded into capillary tubes and imaged with a Visualsonics LAZR photoacoustic scanner from 680 to 900 nm. In Aim 2, mice were injected with 100 μL 0 or 200 U/mL of heparin dissolved in sterile PBS by tail vein. Thirty minutes later, the animals were injected (i.v.) with 100 μL 50 mM methylene blue. Blood was collected via cardiac puncture and imaged within 4 h.
Results: There is a strong correlation between heparin concentration and signal (R^2>0.90) with stability for at least 15 min (Figure 1A) and 2.3 U/mL of sensitivity. A peak near 710 nm is characteristic of the heparin/methylene blue complex (Figure 1B).

The *in vivo* experiments showed a 2.8-fold photoacoustic signal increase in animals treated with MB vs. PBS ($P < 0.0001$) and linear correlation to the aPTT (Figure 2A, B). This approach also has utility with LMWH (Figure 2C) with a detection limit of 0.1 mg/ml. The order of addition was important—animals injected with MB first followed by heparin showed little signal. (Figure 2B).

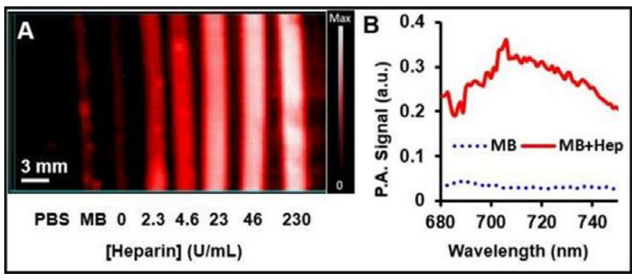

Figure 1

Conclusions: To the best of our knowledge, this is the first report to describe an imaging technique to monitor heparin. The approach could be used to construct a wearable sensor to monitor heparin anticoagulation in real time analogous to the pulse oximeter (Figure 2D).

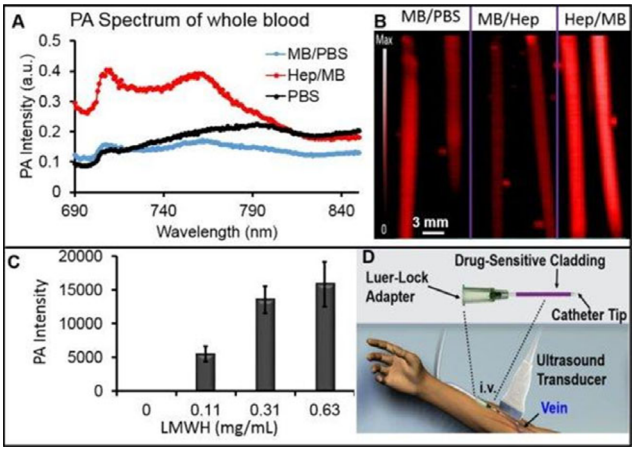

Figure 2

CA17

Inclusion of a heparin neutralizer in the plasma diluent confers specificity to the FXa direct oral anticoagulants rivaroxaban, apixaban, and edoxaban in an anti-Xa chromogenic assay

Goldford M[1], Calhoon W[2], Nguyen D[3], Woodhams B[4] and Morris M[3]

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Background: The anti-Xa chromogenic heparin assay has become a popular method for the measurement of the FXa direct acting oral anticoagulants (DOACs). We hypothesized that including a heparin neutralizer in the plasma diluent for this assay would make the assay specific for the FXa DOACs without altering the dose-response seen in the unmodified assay.
Aims: Determine the dose-response of FXa DOACs in a prototype anti-Xa chromogenic heparin assay with and without a heparin neutralizer in the plasma diluent.
Methods: A prototype anti-Xa chromogenic heparin assay was developed using bovine FXa and the FXa substrate Z-D-Arg-Gly-Arg-pNA, and adapted to the Behnk Compact X clinical coagulation analyzer. Plasma diluent was imidazole buffered saline (IBS) alone or IBS supplemented with 3 ug/mL hexadimethrine bromide (PolybreneTM). DOAC plasma calibrators included 0, 50, 100, 250, or 500 ng/mL of either rivaroxaban, apixaban, or edoxaban. Dose-responses of the OD/min were determined for the calibrators spiked with either 1 U/mL unfractionated heparin (UFH) or 2 U/mL low molecular heparin (LMWH), or vehicle, and diluted with either IBS alone or IBS-polybrene.
Results: In the reactions without any heparins spiked into the sample the assay demonstrated DOAC dose-dependent inhibition of the OD/min with either IBS or IBS-polybrene as the plasma diluent. When the reactions included either UFH or LMWH the dose responses of the assay for DOACs were eliminated with the IBS diluent and restored with the IBS-polybrene diluent. Each DOAC dose response of the assay was equivalent with either 1) no added heparins with IBS diluent, 2) no added heparins with IBS-polybrene diluent, or 3) added heparins with IBS -polybrene diluent.
Conclusions: Including a heparin neutralizer in the plasma diluent in an anti-Xa chromogenic assay provides specificity to the FXa DOACs without altering the assay configuration, eliminating confounding of the heparin effect from the DOAC effect.

CA18

Influence of Factor VII on INR determined with recombinant human and tissue-extract thromboplastins

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Background: Differential sensitivity to clotting factor VII (FVII) has been suggested between recombinant and tissue-extract thromboplastins used for International Normalized Ratio (INR) measurement, but evidence is scarce. Differential sensitivity to FVII is clinically relevant, since it directly affects stability of treatment with vitamin K antagonists (VKA). We measured the change in INR after addition of different doses of FVII to plasma of patients on VKA using three tissue-extract (Hepato Quick, Neoplastin C1 + , Thromborel S) and three recombinant (Innovin, Recombiplastin 2G, Coaguchek XS) thromboplastins.

Aims: To determine if thromboplastins from different sources react differently to a measured change in FVII.

Methods: Three doses of purified human FVII (0.006, 0.012 and 0.062 μg/mL plasma) or buffer (0.15M NaCl, 20 mM Tris.HCl, pH 7.4, 1% BSA) as control, were added to five certified pooled plasmas of patients on VKA (INR 1.5–3.5). INRs obtained for plasmas spiked with Factor VII were compared with the same plasmas spiked with the same volume of buffer without Factor VII. Change in FVII activity was measured with a specific bioassay (Recombiplastin 2G) and relative INR changes in the pooled plasmas after addition of FVII were compared between reagents using the Wilcoxon signed-rank test or Friedman test.

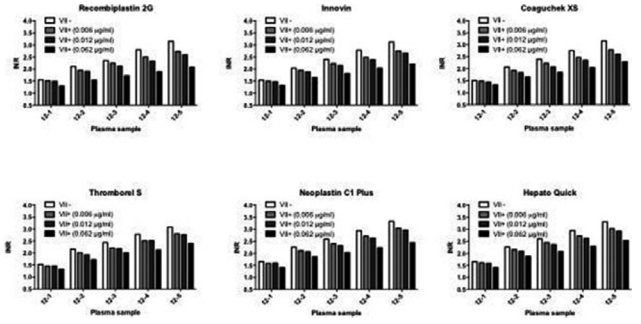

Figure INR results after FVII addition.

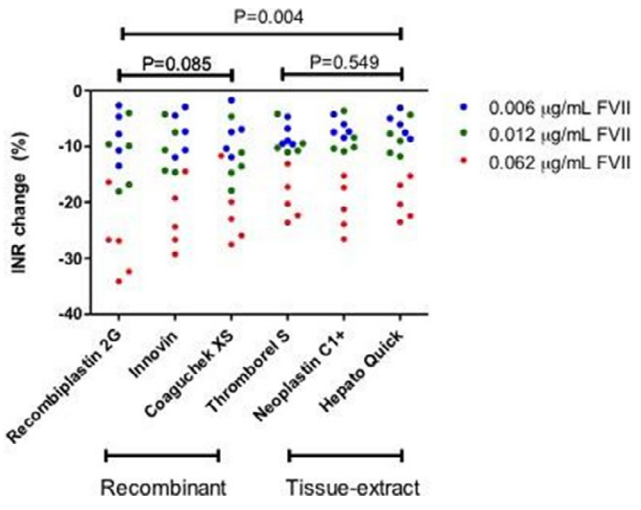

Figure Relative INR change in plasma after FVII addition.

Results: After FVII addition, FVII activity in the pooled plasmas increased by 3.2, 6.4 and 32.0% respectively. All thromboplastin reagents showed dose-dependent INR changes.

The relative INR change significantly differed between the six thromboplastin reagents ($P = 0.004$). No differences were observed amongst recombinant ($P = 0.085$) or tissue-extract ($P = 0.575$) thromboplastins.

Pooled results showed that relative INR changes were significantly greater when measured with recombinant compared to tissue-extract thromboplastins ($P < 0.001$).

Conclusions: Our results indicate that recombinant thromboplastins used for INR measurement are significantly more sensitive to FVII than tissue-extract thromboplastins.

CA19

The effect of dabigatran on thrombin generation *in vitro*

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Background: Dabigatran etexilate, a direct thrombin inhibitor (DTI), is at least as effective as warfarin in the prevention of thrombo-embolic complications of non-valvular atrial fibrillation and in the treatment of venous thrombo-embolism. Since dabigatran has a lower risk of major and intracranial bleeding than warfarin and since there is no need for routine monitoring with blood tests, dabigatran etexilate is increasingly prescribed. However, in several clinical situations, information on the anticoagulant effect of dabigatran is needed. The relationship between plasma concentrations of dabigatran and clinical bleeding and thrombotic events has not yet been fully elucidated. We expect that thrombin generation will reflect the anticoagulant effect of dabigatran.

Aims: To evaluate the effect of dabigatran on thrombin generation *in vitro*.

Methods: Tissue factor-induced thrombin generation assay was measured in dabigatran spiked plasma (0–1000 ng/mL). Dabigatran concentrations were measured using liquid chromatography- tandem mass spectrometry (LC-MS/MS).

Results: The peak height, lagtime and time to peak increased with increasing dabigatran concentrations. The increase in ETP at low concentrations of dabigatran was unexpected but also seen in previous studies with other DTIs. This may be explained by the suppression of thrombin-induced and thrombomodulin-mediated activation of protein C and thrombin activatable fibrinolysis inhibitor (TAFI).

Conclusions: Low levels of dabigatran, within what is currently considered the therapeutic range, enhance thrombin generation *in vitro*. Studies determining the anticoagulant effect of dabigatran in patients are needed. However, in order for ETP to be used for monitoring of patients treated with DTIs, several challenges have to be overcome.

CA20

Commutability of samples used to assess assays for direct oral anticoagulants (DOACS): data from UK NEQAS for blood coagulation multicentre studies

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Background: We have previously reported results from a multicentre exercise in which laboratories performed assays for direct oral anticoagulants (DOACs) Dabigatran, Rivaroxaban and Apixaban. These were on samples spiked with the respective drugs.
Aims: We describe here a further exercise in which we explored commutability of spiked samples and those from patients receiving Dabigatran and Rivaroxaban.
Methods: 2 lyophilised plasma samples were prepared for each DOAC, 1 a normal plasma spiked with drug, kindly provided by the manufacturers, and 1 from a patient receiving treatment with the same drug. UK NEQAS BC centres were asked to perform their routine assay for each DOAC. Median, coefficient of variation (CV) and range were determined for each method and reagent group.
Results: Dabigatran assays (n = 56) - DOAC15:01 (patient sample) median result 92 ng/ml, CV 15.1%; DOAC15:02 (spiked sample) median result 154 ng/ml, CV 16.7%. Similar precision was seen for these two samples, although the pattern of results for the most widely used kits differed (Hyphen Hemoclot median 10 ng/ml lower than HemosIL DTI for DOAC15:01, 5 ng/ml higher for DOAC15:02. This may reflect the different drug concentration in each sample. Rivaroxaban assays (n = 68) - DOAC15:03 (patient sample) median result 140.7 ng/mL, CV 16.5%; DOAC15:04 (spiked sample) median result 140.0 ng/ml, CV 18.2%. Similar precision was seen for these two samples, and the pattern of results for the most widely used kits was the same (Biophen DiXal median 30 ng/ml higher than HemosIL liquid anti-Xa for both samples).
Conclusions: This exercise confirmed variability in results across methods and within method groups. Good comparability was seen between results for spiked and patient samples, especially for Rivaroxaban samples, indicating suitability of spiked samples as quality control material for Dabigatran and Rivaroxaban assays. We plan to perform further similar exercise with other DOACs in due course.

CA21

Is the Dilute Russell's viper venom time (DRVV-T) a useful assay for all direct oral anticoagulants (DOACs)?

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Background: DOACs occasionally may require measurement of anticoagulation intensity in certain clinical situations. Routine coagulation screening assays do not quantify anticoagulation intensity. Therefore, it is necessary a simple and quantitative test that correlates with these drugs concentrations. Dilute Russell's Viper Venom Time (DRVV-T) contains a potent activator of factor X and catalyzes the transformation from prothrombin to thrombin; the presence of factor IIa orXa inhibitors may increase this time.
Aims: To analyze the correlation between DRVV-T and direct oral anticoagulants plasma levels.
Methods: Levels of dabigatran and apixaban were tested in 45 blood samples from 26 patients. Rivaroxaban samples are being processed but results are not definitive yet. To determine the highest and the lowest blood levels of the drugs by obtaining a blood sample just before the morning dose then sampling again 2 h after the administration.
DOACs plasma concentrations were measured using the Direct Thrombin Inhibitor Assay from HemosIL for dabigatran and HemosIL Testing Solution for apixaban. Calibraton curves were obtained from 100 healthy patients. We performed DRVV-T and normal ratio was established by measuring 35 normal samples.
Results: We observed that DRVV-T was prolonged in a concentration-dependent fashion at higher DOACs levels. There was a linear correlation between apixaban levels (ranging between 49.74 ng/mL - 423.47 ng/mL) and ratios for DRVV-T test, Pearson′s correlation value was 0.84 with bilateral significance p value< 0.01. The same results with direct thrombin inhibitor, dabigatran (43.79 ng/mL - 250 ng/mL), with a Pearson′s value of 0.69 ($p < 0.01$). When we analyzed both together, Pearson′s correlation value was 0.62 (p value < 0.01). A cut-off level of 1.2 for DRVVT ratio, corresponds to the presence of more than 30 ng/mL DOAC concentration.
Conclusions: DRVV-T showed a close linear correlation between its ratio and plasma drug concentrations and a cut off ratio of 1.2 can be established.

CA22

Outcome of DOAC exposure during pregnancy (. . .and the problem of event reporting. . .)

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Background: Exposure of pregnant women to vitamin-K antagonists (VKA) carries a high risk for embroypathy of at least 7%, but for direct oral anticoagulants (DOAC) the embryopathy risk is unknown.
Aims: We aimed to evaluate pregnancy outcomes after DOAC exposure using data from various sources.
Methods: Cases of DOAC pregnancy exposure were collected from literature search, from questionnaires sent to German gynecologists, obstetricians and hematologists and from pharmacovigilance databases with the DOAC manufacturers, the German drug authority (BfArM) and the European Medicines Agencies (EMA).
Results: A total of 357 reports were available and, after removing duplicates, 233 definitely separate cases of DOAC exposure in pregnancy could be identified. A main limitation was the lack of details and insufficient follow-up data in the datasets provided by the DOAC manufacturers and authorities.
Information of pregnancy outcome was available in 137/233 cases (58.8%) and consisted of 67 live births (58.9%); 31 miscarriages (22.6%); 39 elective pregnancy interruptions (28.5%).
In 3 cases, the pregnancy was still ongoing. In 93 cases (39.9%) no outcome data were available.
Of the 137 pregnancies with reported outcomes, there were 7 abnormalities (5.1%) of which 3 (2.2%) could potentially be interpreted as embryopathy: life birth with facial dysmorphism; miscarriage with limb abnormality in week 10; elective pregnancy termination due to a foetal cardiac defect in a women with a pregnancy history of Fallot tetralogy.
Conclusions: Currently available data do not suggest a high risk for DOAC embryopathy but case numbers are limited and demonstrate significant data gaps. Therefore, risk assessment needs to continue and pregnancy has to be avoided in DOAC patients. However, currently available data do not justify pregnancy termination just because of DOAC exposure and non-directive counselling as well as close pregnancy surveillance is recommended.

Table 1 Data quality according to data source. (Abstract CA22)

Data source (n)	NOAC indication available	NOAC dosage available	NOAC pregnancy exposure duration available	Pregnancy outcome available
Case collection; n=15	15/15 (100%)	15/15 (100%)	15/15 (100%)	12/15 (80.0) + 3 ongoing
Pharmacovigilance database of NOAC manufacturers.; n= 83	Bayer: 34/47 (72.3%) Pfizer: data declined DS: 10/10 (100%) BI: 16/26 (61.5%)	Bayer: 11/47 (23.4%) Pfizer: data declined DS: 7/10 (70%) BI: 14/26 (53.8%)	Bayer: 0/47 Pfizer: data declined DS: 9/10 (90%) BI: 14/26 (53.8%)	Bayer: 28/47 (59.6%) Pfizer: data declined DS: 10/10 (100%) BI: 12/26 (46.2%)
Pharmacovigilance database BfArM; n=13	Apixaban: 1/1 (100%) Rivaroxaban: 12/12 (100%)	Apixaban: 0/1 Rivaroxaban: 8/12 (66.7%)	Apixaban: 1/1 (100%) Rivaroxaban: 5/12 (41.7%)	Apixaban: 1/1 (100%) Rivaroxaban: 8/12 (66.7%)
Summaries from manufacturers PSUR from EMA; n=196	Bayer: 9/148 (6.1%) Pfizer: 11/21 (52.4%) BI: 0/26	Bayer: 5/148 (3.4%) Pfizer: 0/21 BI: 13/26 (50%)	Bayer: 0/148 Pfizer: 3/21 (14.3%) BI: 0/26	Bayer: 58/148 (39.2%) Pfizer: 8/21 (38.1%) BI: 11/26 (42.3%)
SmPC (edoxaban); n=10	10 (100%)	0/10	0/10	10 (100%)
Literature search - FDA; n=2 - Embryotox; n=39 - case report	Apixaban: 1/2 (50%) Rivaroxaban: 39/39 (100%) Rivaroxaban: 1/1 (100%)	Apixaban: 1/2 (50%) Rivaroxaban: 30/39 (76.9%) Rivaroxaban: 1/1 (100%)	Apixaban: 2/2 (100%) Rivaroxaban: 37/39 (94.9%) Rivaroxaban: 1/1 (100%)	Apixaban: 2/2 (100%) Rivaroxaban: 39/39 (100%) Rivaroxaban: 1/1 (100%)

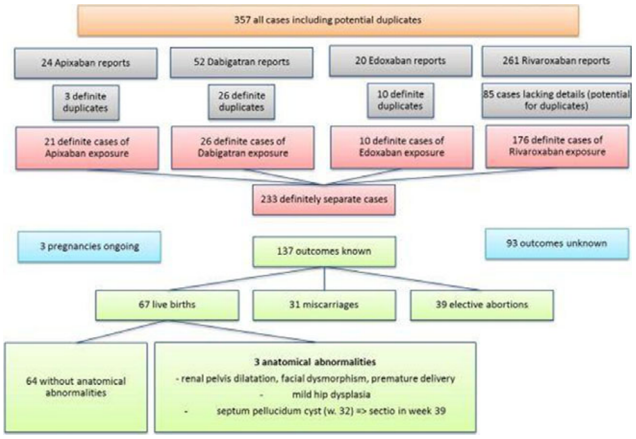

Figure Flow chart of data sources. (Abstract CA22)

CA23

Consequences of warfarin suspension after major bleeding in a cohort of very elderly patients

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Background: Non-valvular atrial fibrillation (NVAF) is very common in elderly. Oral anticoagulant therapy with vitamin K antagonists (VKA) is greatly effective in stroke prevention but may also expose to bleedings, which are more frequent in the elder and frail patients. Decision whether or not to discontinue VKA after a major haemorrhage in such population has not been thoroughly investigated.

Aims: To report the outcomes after a major bleeding, according to persistence or discontinuation of VKA after the index event, in a cohort of very elderly patients with NVAF attending a large size anticoagulation clinic.

Methods: This is a retrospective analysis of an inception cohort study (VENPAF, i.e. Very Elderly NVAF patients naïve to VKA therapy). We selected patients who experienced a major bleeding event during VKA therapy and followed them to report any further event (ischemic or haemorrhagic). Decision to stop the treatment after the index event was also collected.

Results: Major bleeding was reported in 65 out of 798 patients (8%) with an incidence of 3.4%pts/y. 28% of these patients experienced a fatal event and were not considered in subsequent analysis. Of the remaining subjects who experienced a major bleeding, 24 suspended VKA while 25 persisted on treatment. Patients were followed up to 3 years after the event and experienced eight major bleedings and seven ischemic events. All but one ischemic events happened in patients who suspended treatment while major bleedings were equally distributed in both groups. Survival curve analysis (see Figure) showed significantly less events for patients who persisted on VKA.

Conclusions: In a highly selected cohort of very elderly patients who started anticoagulation for NVAF, VKA therapy demonstrated a good risk-benefit profile even in patients who experienced a major bleeding during anticoagulation. Current antithrombotic treatment in this class of very elderly, fragile and complex patients is still a challenge.

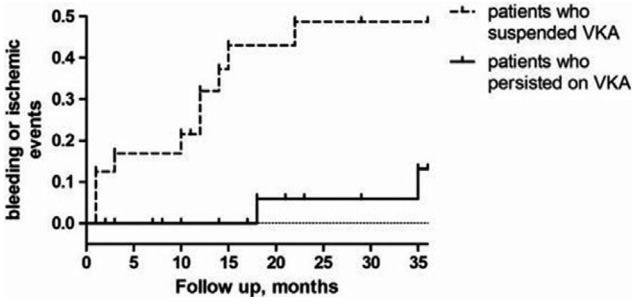

Figure Event-free survival according to VKA persistence.

CA24

Assessment of low molecular weight heparin accumulation in patients with chronic kidney disease: results from the TRIVET study

Lim W[1], Crowther M[1], Wang L[2], Douketis J[1], Schnurr T[2], Moreau C[2], Clase C[1], Rodger M[3], Yeo E[4], Leblanc M[5], McFarlane P[4] and Ribic C[1]

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Background: The predominant renal elimination of low molecular weight heparin (LMWH) has raised concerns regarding the safety of therapeutic dose LMWH in patients with renal impairment. Tinzaparin may be less dependent on renal clearance compared to other LMWHs. Anti-Xa levels are a surrogate measure of LMWH activity levels and bleeding risk.

Aims: To assess for tinzaparin accumulation in patients with venous thromboembolism (VTE) and varying degrees of renal impairment using repeated trough anti-Xa levels.

Methods: Prospective multicenter cohort study of patients with objectively confirmed VTE treated with tinzaparin (Tinzaparin in Renal Insufficient Venous Thromboembolism (TRIVET) study; NCT00186745). Informed consent was obtained and the study approved by the ethics board at each site. Patients were stratified into 4 groups based on calculated Cockcroft-Gault creatinine clearance (CrCl) (ml/min): >60, 30–60, < 30 and hemodialysis (HD)-dependent. Tinzaparin 175 IU/kg was administered subcutaneously once daily for up to 7 days. Trough anti-Xa levels were measured twice, prior to the 3rd – 5th dose and prior to the 5th – 7th dose of tinzaparin. Accumulation was defined as anti-Xa >0.5 IU/mL which resulted in tinzaparin dose reduction. Bleeding and recurrent thrombotic events were recorded.

Results: 148 patients were enrolled from 5 centers (Table 1). Using CrCl >60 as the comparison, mean trough anti-Xa levels were significantly higher in CrCl < 30 and HD-dependent patients ($P < 0.005$). The number of patients with accumulation and bleeding was most evident with CrCl < 30. There were 5 major bleeding events occurring within 7 days of tinzaparin administration (none with anti-Xa >0.5 IU/mL) and 1 recurrent VTE (CrCl >60) (unadjudicated).

Table 1

Cockcroft-Gault CrCl (ml/min)	*N*	Mean anti-Xa ± SD (IU/mL)	Patients with anti-Xa >0.5 IU/mL, N (%)	Major bleeding, N (%)
>60	56	0.16 ± 0.12	4 (7.1%)	0
30–60	38	0.20 ± 0.17	4 (10.5%)	1 (2.6%)
<30	29	0.29 ± 0.23	9 (31.0%)	2 (6.9%)
HD-dependent	25	0.34 ± 0.28	7 (28.0%)	2 (8.0%)

Conclusions: Therapeutic dose tinzaparin is associated with higher mean trough anti-Xa levels in patients with CrCl < 30 and HD-dependent patients. Major bleeding events were more frequent in patients with CrCl < 30 but did not appear to correlate with trough anti-Xa levels >0.5 IU/mL.

CA25

Major bleeding in patients with atrial fibrillation on non vitamin k antagonist oral anticoagulants - data from Ljubljana registry

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Background: The use of non vitamin K antagonist oral anticoagulants (NOAC) for the prevention of stroke is increasing in patients with atrial fibrillation (AF), however data on bleeding complications in routine clinical practice are scarce.

Aims: To assess bleeding complications on NOAC in AF patients treated in community-settings.

Methods: Using data from prospective patient registry at a high-volume anticoagulation clinic in a tertiary medical centre we analysed major bleeding rates in patients with AF newly stated on NOAC in the period from September 2012 to September 2015. Major bleeding was defined as haemoglobin drop ≥20 g/L, need for transfusion, hospitalisation or invasive procedure.

Results: From total of 1863 patients who started on NOAC, 863 patients were treated with dabigatran (age 72 ± 8, $CHADS_2$ score of 2.0 ± 1.1), 760 patients with rivaroxaban (age 78 ± 8, $CHADS_2$ score of 2.3 ± 1.1) and 240 patients with apixaban (age 80 ± 7, $CHADS_2$ score of 2.0 ± 1.1). The mean treatment duration was 16 ± 10 months in patients on dabigatran, 15 ± 8 months in patients on rivaroxaban and 4 ± 2 months on apixaban. Major bleeding occurred in 53 patients, in 19 patients (1.65 events per 100 patient-years) on dabigatran, in 28 patients (2.76 events per 100 patient-years) on rivaroxaban and in 6 patients on apixaban. Bleeding complications were predominantly gastrointestinal (57%). Of 14 patients with an intracranial bleeding, 5 episodes occurred after trauma. Only in 3 patients treatment with prothrombin complex concentrate was needed, 3 patients died (2 patients with intracranial and 1 patient with gastrointestinal bleeding). Compared to patients without bleeding, patients with bleeding were older (77 ± 8 vs 79 ± 8 years) and had higher $CHADS_2$ (2.3 ± 1.1 vs 2.6 ± 1.1) and HASBLED score (1.0 ± 0.6 vs 1.3 ± 0.6) (all $P < 0.05$).

Conclusions: Our data contribute to the confirmation of low bleeding rates on NOAC in unselected AF patients in routine clinical practice.

CA26

Patients anticoagulation knowledge (VKA) and INR goal attainment-comparison 2014 and 2016

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Background: Successful anticoagulation treatment (vitamin K antagonists- VKA) is a long standing challenge and it depends upon:

- the patient knowledge for the treatment
- Patient adherence to treatment
- doctor patient relationship

From July 2013 MOH introduced project "admission time" Each patient has a reserved scheduled time of about 10 min to spend with his doctor that aloud an enough time to educate and improve doctor-patient. relationship.

Aims:

- To compare patient anticoagulation knowledge between 2014 - January and 2016- January (after the intervention)
- To examine a relationship between patients anticoagulation knowledge and anticoagulation control, INR goal attainment

Methods: Upon voluntary consent a 20 questions multiple choice questionnaire was given to the patients who attend our clinic in 2 consecutive days in January 2014 and 2016. Patients were on VKA treatment more than 5 months.

Passing score: 14 correct answers (CA) of 20 questions (Q).

Demographics data, PT INR results of 3 previous visits, were collected from the patient records: Knowledge scores were compared using Student t test analysis, INR control was defined by number of INR within

therapeutic range and standard deviation (SD), Bevariete analyses of INR control with anticoagulation knowledge assessed with Spearman's rho correlation.

Table 2

Demographics characteristics	2014	2016
No of patients	66 patients	72 patients
Age: >50; 51–60; 61–70; 71–85;	12% 13% 18% 57%	14% !6% 17% 53%
Gender: Male/Female	39%/61%	42%/58%
Education: Analphabet; Elementary; High school; University	1% 39% 45% 15%	2% 40% 46% 12%
The majority of patients were undergoing anticoagulation treatment for I 48, I42, I49, DVT/PE, and after cardiovascular surgery Z95.x, For most of them INR goal is 2–3 (82–83%) other 25–3 (18–17%)		INR PT (18–35): 2014 (62,5%) : 2016 (68,1%)

Results: Out of 80 patients, 66 completed the questionnaire in 2014 (45% passing score) and 72 in 2016 (77% passing score).
Student t test paired analysis shows very significance - p less 0,0024 on improvement of patient knowledge.
Spearman's rho correlation $R = 0{,}250183$. Two tailed $P = 0{,}2177$ were not statistically significant.

Table 1 Question number (Q No): Correct answers %.

	Q No	CA % 2014	CA% 2016	Student t -test paired
Passing score > 14 CA ($P < 0{,}0005$)		45%	77%	**Two tailed p value less 0,0024 very statistically significant**
Medication-medication administration interactions	1 4 8 14 17 18	69 78 57 74 51 67	77 92 62 85 65 85	CI 95% : -15.98–4.02 Intermediate values used in calculation $t = 3.5000$
Diet	2 5 6 16	36 45 36 57	42 65 61 57	Df=19 Standard error of difference 2,857
Side effects	3 11 15	67 12 87	65 27 87	2014 Mean 61,55: SD 21,09; SEM 4,72 N20
Informing health care providers (doctor patient relationship) Laboratory monitoring Activity Pregnancy	7 9 10 12 13 19 20	75 88 85 79 84 36 48	**100 96 100 96 85 65 19**	2016 Mean 71,55; SD 23,23: SEM 5,19 N20
	2014	2016		
PT INR SD	Mean 1,80 SD 0,667 Variance 44,59	Mean 1.99 SD 0,679 Variance 46,15		**Spearman's rho correlation $R = 0{,}250183$/p Two tailed p = 0,2177 not statistically significant**

Conclusions: Results show that knowledge about VKA exert significant influence on medication adherence and doctor- patient trust, but yet this still not predict therapeutic anticoagulation control. Improvement of the anticoagulation knowledge in patient improves compliance and control, but medication adherence did not predict therapeutic anticoagulation level.

CA27

Can PT, aPTT and TT reliably be used to detect DOAC at clinically relevant concentrations in emergency situations? results from a 660-patient cohort study

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Background: Direct oral anticoagulant (DOAC) level may be useful to manage patients receiving rivaroxaban (Riv), apixaban (Api) or dabigatran (Dab) in emergency situations. In many centers, prothrombin time (PT), activated partial thromboplastin time (aPTT) and thrombin time (TT) are the only available tests to detect DOAC presence.
Aims: To evaluate performances of PT, aPTT and TT alone or combined in ruling out relevant concentrations of Riv, Api or Dab (30, 50, 100 ng/mL).
Methods: We conducted a 13-month observational study in 2 university hospitals. We included all consecutive patients receiving Riv, Api or Dab, for whom a DOAC measurement combined with routine tests (PT - Neoplastin® CI+ Stago, aPTT T-coag-Triniclot aPTT®, or TT Thrombin-Stago) had been ordered. DOAC levels were measured using STA-Liquid anti-Xa® (Stago) (Riv and Api) and Hemoclot DTI® (Hyphen) (Dab) with dedicated calibrators. We determined sensitivity (Se), specificity, positive and negative predictive values (NPV) of PT ratio (reference value ≤ 1.30), aPTT ratio (reference value ≤ 1.20) and TT ratio (reference value ≤ 1.30) to detect DOAC levels below 30, 50 or 100 ng/mL thresholds.
Results: We analysed 813 samples, drawn randomly *vs* drug intake as a consequence of the emergency setting, from 660 patients (371 M, 289 F - mean age 64 ± 18 yrs), receiving Riv, Api or Dab. Se and NPV of PT, aPTT, alone or combined, are summarized in Table 1 (see next page). Combined normal PT and aPTT allowed excluding Riv level ≥ 100 ng/mL in 100% of patients, but failed to detect levels up to 50 ng/mL with a satisfactory NPV. NPV did not exceed 70.6% for a 100 ng/mL Api level. In contrast, a 100% NPV was reached for Dab with TT for a threshold as low as 30 ng/mL.
Conclusions: PT and aPTT, even combined, are unreliable to detect Riv concentrations below 30 or 50 ng/mL in a substantial number of patients even though sensitive reagents are used. We confirm that PT and aPTT are useless to predict Api levels. TT is reliable to rule out Dab levels as low as 30 ng/mL.

CA28

Effectiveness of implementing an extended INR testing interval for stable warfarin patients: results from the MAQI² collaborative of anticoagulation clinics

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Table 1 (Abstract CA27)

DOAC (number of samples) (n)		Rivaroxaban (n = 633)			Apixaban (n = 108)			Dabigatran (n = 72)	
Test	Perfomances 95%CI (Agresti-Coull test)	30 ng/mL	50 ng/mL	100 ng/mL	30 ng/mL	50 ng/mL	100 ng/mL	30 ng/mL	50 ng/mL
PT	Sensitivity (%) (95% CI)	74.3 (69.7–78.3)	88.9 (84.9–92.0)	100 (97.6–100.0)	50.6 (40.0–61.2)	52.1 (40.7–63.3)	63.6 (48.8–76.3)	85.4 (71.2–93.5)	87.2 (72.8–94.9)
PT	Negative predictive value (%) (95% CI)	64.7 (59.1–70.0)	88.4 (84.1–91.6)	100 (98.4–100.0)	33.3 (22.7–46.0)	43.3 (31.6–55.9)	73.3 (60.9–83.0)	80.0 (62.3–90.9)	83.3 (66.0–93.1)
aPTT	Sensitivity (%) (95% CI)	46.7 (41.9–51.6)	55.5 (49.9–61.0)	68.4 (61.5–74.6)	41.3 (31.1–52.2)	37.1 (26.8–48.9)	41.9 (28.4–56.7)	95.1 (83.0–99.5)	100.0 (89.3–100.0)
aPTT	Negative predictive value (%) (95% CI)	47.0 (42.2–51.9)	65.8 (61.0–70.2)	85.0 (81.2–88.2)	32.9 (23.0–44.5)	37.1 (26.8–48.9)	64.3 (52.6–74.5)	90.5 (69.9–98.6)	100.0 (81.8–100.0)
Combined PT and aPTT	Sensitivity (%) (95% CI)	77.2 (72.8–81.1)	91.1 (87.3–93.8)	100.0 (97.6–100.0)	58.2 (47.2–68.5)	57.1 (45.5–68.1)	65.1 (50.1–77.6)	97.6 (86.3–100.0)	100.0 (89.3–100.0)
Combined PT and aPTT	Negative predictive value (%) (95% CI)	65.2 (59.2–70.8)	89.5 (85.0–92.7)	100 (98.2–100.0)	35.3 (23.6–49.1)	41.2 (28.7–54.9)	70.6 (56.9–81.4)	94.1 (71.1–100.0)	100.0 (78.4–100.0)

Background: Warfarin-treated patients traditionally have INRs checked at least every 4 weeks. Based on randomized trial data, guidelines support the use of extended INR testing intervals of up to 12 weeks in stable patients.
Aims: To assess the effectiveness of implementing an extended INR testing interval for stable warfarin-treated patients.
Methods: Uncomplicated warfarin-treated patients at six anticoagulation clinics who had stable INR values and warfarin dosing for at least 10–24 weeks were eligible for extended INR testing (up to 6–8 weeks between INR tests). The number of eligible patients and the rate of extended INR testing utilization were assessed quarterly in 2014 (IRB approved). Follow up INR values were compared between eligible patients who did and did not receive an extended INR testing interval.
Results: Of the 3221 patients in our cohort, 644 (20.0%) had stable INRs and warfarin dosing and were eligible for an extended INR testing interval and 380/644 (59%) had their INR interval extended 941 times. Extended INR testing interval patients more often had atrial fibrillation (65.5% vs. 55.3%, $P = 0.01$) and less often had venous thromboembolism (24.2% vs. 32.6%, $P = 0.02$) than eligible patients with standard INR testing intervals (up to 4 weeks). Eligible patients using an extended testing interval had a larger median number of days between INR values (42) than eligible patients with a standard testing interval (28; $P < 0.01$). The percent of eligible patients whose INR testing interval was extended beyond 4 weeks increased from 39.1% in 2014/Q1 to 58.6% in 2014/Q4 ($P < 0.001$ for trend; see Figure). Equal numbers of next INR values were out of range (28.5% vs. 26.0%; $P = 0.73$) as well as bleeding and thromboembolic events were documented between the eligible patients with a standard testing interval and an extended testing interval.

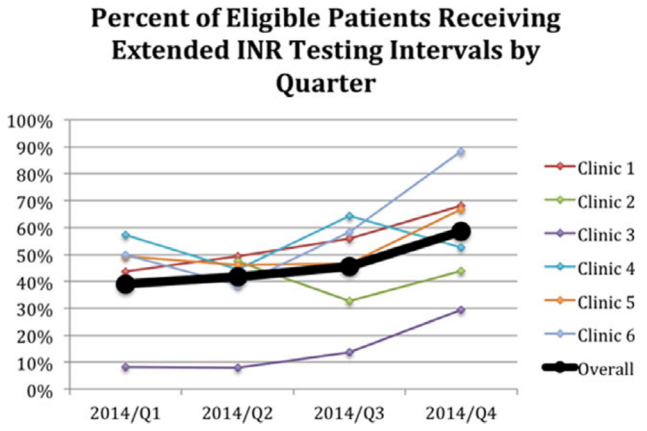

Figure Use of Extended INR Testing Interval by Quarter.

Conclusions: A concerted implementation effort can increase the adoption of an extended INR testing interval for stable warfarin patients without safety concerns.

CA30

estimation of rivaroxaban plasma concentrations in the perioperative setting with or without heparin bridging: an *ex vivo* study using dedicated chromogenic assays

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Background: An estimation of the rivaroxaban plasma concentration before an invasive procedure might be requested for patients requiring a bridging therapy with low-molecular weight heparins (LMWH).
Aims: To assess the performance of the STA®-Liquid Anti-Xa assay (Diagnostica Stago®), and the low and high procedures of the Biophen®DiXaI assay (Hyphen BioMed®), using real-life samples from patients bridged or not with LMWH.
Methods: Seventy-nine blood samples were collected from patients on rivaroxaban at C_{TROUGH} or before an invasive procedure. An estimation of rivaroxaban concentrations was performed with Biophen®DiXaI, Biophen®DiXaI LOW and STA®LAX and compared with the liquid chromatography coupled with mass spectrometry (LC-MS/MS) measurements. Stratifications were performed according to heparin bridging: groupe A (no bridging), group B (bridging) and group C (therapeutic bridging with last administration ≥ 24 h). The anti-Xa activity of LMWH was measured with Biophen®Heparin LRT in group C when rivaroxaban concentration was measured between 0 and 1 ng/ml with LC-MS/MS.

	Group A (n=31)			Group B (n=48)			Group C (n=26)		
	Rivaroxaban plasma concentration (ng/ml)								
Blood samples characteristics	*Range*	*median*	*interquartile range*	*Range*	*median*	*interquartile range*	*Range*	*median*	*interquartile range*
	2.1 to 108.2	32.6	13.4 to 71.3	<1.0 to 10.1	1.0	0.0 to 1.0	<1.0 to 10.1	0.5	0.0 to 1.0
Dedicated chromogenic assays	Bland-Altman analysis (ng/ml)								
	mean bias	*SD*	*95% CI*	*mean bias*	*SD*	*95% CI*	*mean bias*	*SD*	*95% CI*
Biophen®DiXaI	8.8	16.8	-24.1 to 41.8	12.4	9.7	-6.6 to 31.5	11.4	10.4	-8.9 to 31.7
Biophen®DiXaI LOW	4.7	8.4	-11.8 to 21.2	13.5	14.5	-14.8 to 41.8	10.2	7.0	-3.6 to 24.0
STA®-Liquid Anti-Xa	-1.5	10.7	-22.4 to 19.5	20.9	12.6	-3.7 to 45.5	17.6	6.6	4.7 to 30.6

Figure 1

Results: For **Group A**, the Spearman r was 0.91, 0.97 and 0.96 and R-square was 0.82, 0.94 and 0.95 for Biophen®DiXaI, Biophen®DiXaI LOW and STA®LAX, respectively. For **Group B** and **C**, Spearman r and R-square reflected poorly the correlations between chromogenic assays and LC-MS/MS measurements. Agreements with Bland-Altman analyses are demonstrated in Figure 1.
Interferences of residual LMWH anti-Xa activity on Biophen®DiXaI, Biophen®DiXaI LOW and STA®LAX are demonstrated in Figure 2.

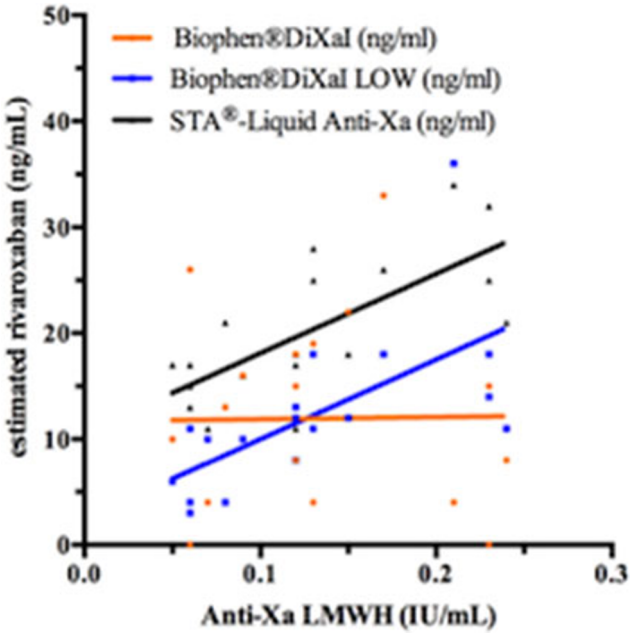

Figure 2

Conclusions: In patients not bridged with LMWH, Biophen®DiXaI LOW and STA®LAX should be preferred for the estimation of rivaroxaban concentrations < 50 ng/mL. In contrary to the Biophen®DiXaI assay, these procedures are sensitive to low residual LMWH activity. Therefore, a chromogenic anti-Xa assay specific to rivaroxaban and efficient in the low ranges is still needed for the perioperative context of patients bridged with LMWH.

CA31

Self-testing and SELF-management of oral anticoagulation therapy (OAT) in children with congenital heart disease (CHD). from the bedside to home. A pilot study in spain

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Background: OAT self-control has shown an improvement in the time in therapeutic range *(TRT)* with a significant reduction in thromboembolic and bleeding events. Children and adolescents on OAT, present also other special challenges in terms of rapid fluctuations in International Normalised Ratio (INR) values and interruption in daily life due to frequent hospital visits. Limited data are available on the safety and efficacy of this modality of anticoagulation control in children with CHD.
Methods: During 2015, a pilot study on coumarin self-control was initiated. This single centre prospective clinical study was designed to evaluate the safety, efficacy and quality of life of a home OAT monitoring with a CoaguChek XProÒ system in paediatric population with CHD, mostly mechanic heart valves (*MHV*). The programme development was structured in three parts: cardiology department doctors and nurses education, families and children′s training and patients clinical follow up. New technology support (*e-mail and WhatsApp*) was used for brief doubts resolution and the all families has access to a web-application to introduce the INR results.
Results: Out of the 20 patients screened, 15 were eligible and accepted to enrol in the study, 47,7% were girls and 53,3% boys. The median age was 8 years (range: 8 months-17 y). 13 patients were anticoagulated for: 8 mitral and 4 aortic *MHV*, 2 for other *CHD* and 1 child for recurrent venous thromboembolism. Cases were vitamin K antagonist naïve. At 6 months of follow up, adherence was good, TRT is superior to 70%. There were not thrombotic or major haemorrhagic events, and all the families and children were satisfied with the improvement of quality of life.
Conclusions: The primary results of this study suggest that self-control of OAT shows a net benefit clinical outcome as first option of coumarin-management. Also the reduction of outpatient visits showed a high level of parents and children′s satisfaction and an improvement in their quality of life.

CA32

Venous thromboembolism treatment with rivaroxaban in adolescents - preliminary findings from the prospective dresden NOAC registry (NCT01588119)

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Background: Venous thromboembolism (VTE) is not uncommon in adolescents and, while direct oral anticoagulants (DOAC) such as rivaroxaban are not approved to treat VTE in under-aged patients, they may offer advantages over alternatives such as vitamin K antagonists or heparin.
Aims: Off-label DOAC use has been reported but the effectiveness and safety in adolescent VTE patients remains to be established.
Methods: Evaluation of clinical outcomes in a prospective cohort of adolescent VTE patients treated with rivaroxaban in the Dresden NOAC registry (all patients and parents provided informed consent for off-label treatment). Patients receive quarterly visits by the registry office. All potential outcome events were centrally adjudicated using standard scientific definitions.
Results: So far, 18 patients < 18 years were enrolled (15 female, 3 male; mean age 15.7 years; mean BMI 22.8 ± 2.8 kg/m²). All patients received rivaroxaban for a first episode of VTE. 8 patients (44.4%) had a family history for VTE. All female patients received hormonal contraception at time of VTE diagnosis. Median time between diagnosis and initiation of rivaroxaban was 25 days (25th/75th percentile 8.5; 122d). During follow-up (mean 580 ± 352d), 2 recurrent VTE events were observed, both of which were related to underexposure. See Table.

Table Cases of recurrent VTE.

Gender	Age [y]	VTE index event	Contraception at time of VTE	Family history of VTE	Predisposing condition	Initial therapy	Time from VTE diagnosis to start rivaroxaban [d]	Duration of rivaroxaban therapy [d]	VTE recurrence (time since VTE diagnosis)
w	17	Proximal DVT and clinically suspected low-risk PE	contraceptive pill	negative	heterozygous factor V mutation, May-Turner-Syndrome, extensive venous stenting	LMWH/VKA	6	> 827 d (ongoing)	13 d recurrent DVT; suspected anticoagulation gap due to vomiting
w	16	Proximal DVT	contraceptive pill	positive	protein C deficiency	LMWH	369	> 566 d (ongoing)	553 d recurrent DVT during long-term rivaroxaban treatment with 1x2.5 mg daily due to bleeding complications

Furthermore, 11 bleeding events occurred (ISTH: 7 minor; 4 clinically relevant non-major bleeding, no major bleeding). 6 patients had a scheduled end of treatment between month 3 and 18, the remaining 12 patients are continuing rivaroxaban.
Conclusions: Rivaroxaban treatment for VTE seems feasible also in adolescent patients with low complication rates and good tolerability over a long period of time. However, a careful patient selection and education is needed at the beginning. VTE treatment of under-aged patients should be performed in specialized anticoagulation clinics and off-label use of NOAC should be limited to cases unsuitable for standard treatment.

CA33

In vivo and *ex vivo* effect of various rivaroxaban concentrations on thrombin generation

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Background: Rivaroxaban (RVX), a direct inhibitor of factor Xa, is used for treatment and prevention of venous thromboembolic events. An unsolved question is the anticoagulant efficacy of a given RVX concentration and its potential hemorrhagic risk related to surgical or pharmacological interventions.
Aims: To assess the ***in vivo*** anticoagulant effect of various RVX concentrations.
Methods: Blood samples from 12 obese patients receiving a single dose of 10 mg RVX before bariatric surgery were obtained at baseline and at different time points over 24 hrs. The anticoagulant effect was assessed *in vivo*, by monitoring thrombin-antithrombin complexes (TAT) and prothrombin fragments 1 + 2 (F1 + 2), and *ex vivo* by measuring tissue factor induced thrombin generation (TG) by calibrated automated thrombogram in patients' platelets poor plasma.
Results: ***Ex vivo*:** The highest inhibition of TG (>70%) was observed at C*max* (120 ng/ml) and it stayed at a similar level at RVX concentrations of 80–90 ng/mL. The degree of *ex vivo* TG inhibition declined to 50–60% with RVX concentrations of 50–60 ng/mL, to 30-35% at RVX 30 ng/mL, and to 15–18% at RVX 15 ng/ml.
In vivo: TAT and F1-2 values significantly decreased at C*max*. During RVX plateau (80–90 ng/mL), a further significant decrease for both activation markers was observed. The anticoagulant effect reached a steady state at drug concentrations of 60–50 ng/mL. TAT and F1 + 2 increased when RVX concentration dropped below 50 ng/mL.
Conclusions: The pattern of *ex vivo* inhibition of TG by RVX significantly differs between concentrations of 80–120 ng/ml, 50–60 ng/mL, 30 ng/mL, and 15 ng/mL. TG is progressively inhibited *in vivo* by steady-state RVX concentrations of 80–90 ng/mL. This is no longer the case when RVX falls below 60 ng/ml. Of note, at 30 ng/ml RVX is still able to inhibit TG *ex vivo* but not *in vivo*. These data show that *ex vivo* TG inhibition does not correlate with the *in vivo* effect and they contribute to define the *in vivo* anticoagulant efficacy of a given plasmatic RVX concentration.

CA34

Evaluation of the CP3000 coagulation analyzer

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Background: The CP3000 is a routine coagulation analyzer for average and large sized laboratories. It is, combined with a range of reagents for clotting (PT, aPTT, fibrinogen, thrombin time), chromogenic (antithrombin) and immunoturbidimetric (D-dimer, FDP) assays, manufactured by Sekisui Medical and launched in Europe by Abbott.
Aims: To investigate the analytical performance and practical usability of the CP3000.
Methods: The CLSI H57 protocol was used as a basis to study precision (N = 20 or higher), carry-over, reagent stability and sensitivity, reference ranges and potentially interfering factors.on all available assays.
Results: Within-run and total CV were maximal (based on three levels) 1.1 and 2.2% for PT, 2.3 and 2.8% for aPTT, 4.2 and 4.5 for thrombin time, 4.2 and 7.1% for antithrombin, 5.2 and 7.4 for fibrinogen, time 3.5 and 4.3%, 12.2 (12.6% at clinical cut-off) and 15.0% for D-Dimer and 23.8 (at low level of 2.0 mg/L) and 26.5% (both at low level of 2.0 mg/L) for FDP, respectively.
Statistically significant sample and reagent carry over issues were demonstrated, which were however clinically not relevant. For example aPTT before and after a fibrinogen test 31.8s and 32.0s, respectively.
Reagent on-board stability was satisfactory, ranging from 30 h for antithrombin to 582 h for D-dimer.
Reference ranges were for PT 10.6–13.4s (N = 191) and aPTT 25.6–38.4s (N = 177).
Sensitivity of the aPTT and PT reagents to factor deficiencies was established with prolongation of the clotting time at 10–20% and 20–40% deficiency respectively.
Conclusions: The CP3000 and associated reagents are easy to handle. The system is small and very fast when measuring coagulation based and chromogenic assays, but slower for the immunoturbidimetric assay methods. Its analytical performance meets medical needs and is well suited for use in a clinical laboratory.

CA35

Evaluation of the intra-, inter- and day-to day variability of the ROTEM®

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Background: ROTEM® is a point of care device that measures real time global clot formation and dissolution in whole blood.
Aims: The aim of this study was to assess the intra- and the interindividual variability and the circadian variation in ten healthy subjects over a period of 8 weeks.
Methods: Blood samples were taken into citrated vacutainer tubes at the following time points: day (d) one 8 AM, 11 AM, 2 PM, d7, d14, d21, d35, d42, d49 and d56. The blood samples were analysed by using the NATEM® test system. Four standard parameters were assessed: the clotting time (CT), clot formation time (CFT), maximum clot firmness (MCF) and the maximum lysis (ML).
Results: The intraindividual coefficient of variation (CV %) ranged for the clotting time (CT) from 11% to 23% (mean: 16%) and for the CFT from 14% to 39% (mean: 25%). The MCF within single

volunteers was fairly constant with a CV% of 7% (4% to 26%), whereas the maximum lysis showed more variability (range 8%-44%, mean 20%). Interindividual variability was estimated by the coefficient of variation for each day. The CV% for MCF was 10% (5%-25%), whereas ML showed more variability with a CV% of 29% (19%-71%). Within one day, a statistically significant circadian variability could be observed in all individuals. Compared to morning values, CT, CFT and the sum of CT + CFT were significantly shortened in the afternoon. The maximum clot firmness and the lysis however did not change noticeably (Figure 1).

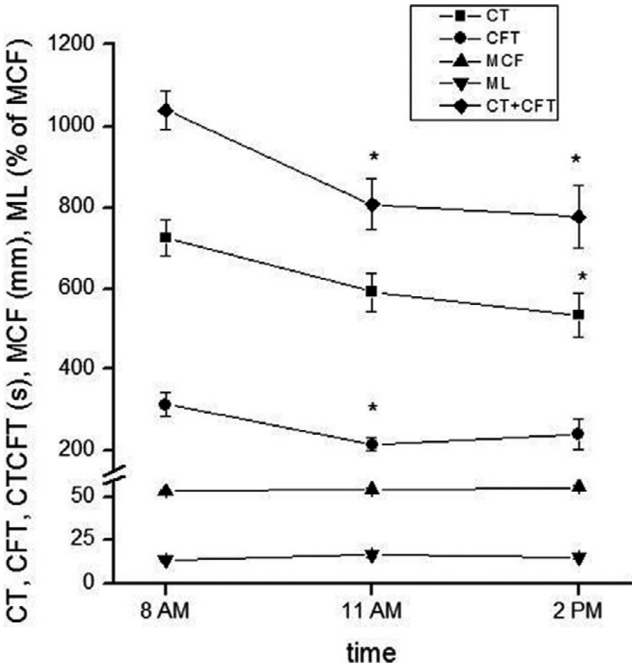

Figure 1

Conclusions: Due to the high intra- and interindividual variations we recommend duplicate measurements. In terms of diurnal variation, all parameters significantly peaked in the morning, indicating decreased coagulation processes in the morning. However, this might be due to the high intra- and interindividual variations and the small sample size.

CA36

Quality assurance in a haemostasis laboratory - an evaluation of 7 year quality indicator data utilizing sigma metrics

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Background: Around two-thirds of important clinical decisions about the management of patients are based on laboratory test results. Clinical laboratories are required to adopt quality assurance (QA) measures to ensure provision of accurate and precise results. Most clinical laboratories monitor performance by defining a set of quality indicators. Performance evaluation on sigma scale is relatively a newer concept in laboratory medicine which provides more meaningful interpretation and hence prospects of improvement in clinical laboratories.

Aims: The aim of this study was to determine performance of a coagulation laboratory by converting the frequency of errors to the sigma scale.

Methods: Seven-year quality indicator data of a coagulation laboratory was evaluated. Frequency of errors from pre-analytical to post-analytical phases of testing process was calculated and converted to the sigma scale. An indicator with a Sigma value of ≥4 was considered acceptable but a process for which the Sigma value was ≥5 (i.e. 99.977% error-free) was considered good performance.

Results: In the seven-year period, a total of 890535 specimens were received in the laboratory. Total error rate was 0.04% and of all the quality indicators used in this study the average Sigma level was 4.9. Acceptable sigma value of ≥4 was achieved for most indicators; two indicators – failure to inform critical results and delay in stat reporting – were below 4 on the sigma scale.

Conclusions: Utilization of sigma metrics is a more effective way of monitoring quality and hence improving efficiency in a clinical laboratory.

CA37

Propagation of thrombin generation in space reveals difference in effects of heparin, dabigatran and rivaroxaban

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Background: Difference in efficiency between anticoagulants and suitable methods of their monitoring are now matters of debate.

Aims: Developing an assay for monitoring hemostasis system state during therapy with traditional and direct oral anticoagulants that can reflect the mechanism of action of each individual drug.

Methods: Plasma from healthy individuals was spiked in-vitro with unfractioned heparin (UFH), dabigatran and rivaroxaban. We used a fluorogenic substrate-based method Thrombodynamics-4D, that allows monitoring of propagation of thrombin generation in space from a tissue factor (TF) coated surface in a thin layer of plasma and simultaneously fibrin formation. Spatio-temporal distribution of fluorophore was transformed into thrombin distribution in time and space.

Results: Generation of thrombin in normal plasma first appears on the activating surface as a rapid burst of thrombin with subsequent decay. Thrombin then propagates from the surface as a stationary peak. Spatial thrombin distribution is characterized by the lag-time of thrombin formation, height of thrombin peak near the activator and the stationary moving peak and rate of thrombin propagation that is equal to the rate of fibrin clot growth. The table (see next page) shows how the parameters of thrombin distribution change in presence of different anticoagulants compared to control.

Heparin fully inhibited formation of moving peak of thrombin without affecting the initiation time. In contrast, dabigatran significantly increased initiation time and decreased synchronically the height of thrombin peak on the activator and in space. Remarkably, propagation of thrombin in space was only slightly inhibited. Thrombin propagated as a peak of lower height, but there was no change in its shape. Rivaroxaban delayed thrombin formation and lead to fast transformation of moving peak to a plateau.

Conclusions: Spatial distribution of thrombin is sensitive to UFH, dabigatran and rivaroxaban and reveals differences in their effects on the coagulation system.

Table Change of thrombin distribution parameters. (Abstract CA37)

	UFH (0.15 IU/ml, $n = 10$)	Dabigatran (0.3 μM, $n = 12$)	Rivaroxaban (0.3 μM, $n = 9$)
Lag-time	0%	+ (50 ± 47) %	+ (130 ± 100) %
Thrombin peak height (10 min)	- (40 ± 10) %	- (37 ± 12) %	- (34 ± 13) %
Moving thrombin peak height (60 min)	-100%	- (44 ± 7) %	- (70 ± 16) %
Rate of clot growth	- (55 ± 4) %	- (6 ± 4) %	- (19 ± 4) %

CA38

A performance evaluation of a novel human recombinant tissue factor prothrombin time reagent

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Background: We report on the performance of a novel prothrombin time reagent (NewPT) which utilises human recombinant tissue factor produced by silkworm technology and synthetic phospholipids. Insect systems are widely used to produce proteins from higher eukaryotes because they have a similar pattern of glycosylation, phosphorylation, and protein processing.
Aims: The aim of this study was to compare the performance of NewPT with two widely used PT reagents.
Methods: The performance of NewPT (Sysmex Corp. Japan) was compared to that of two commercial PT reagents containing either recombinant human TF (A) or human placental thromboplastin (B) on a fully automated coagulometer. Analyser specific ISI values were determined using Technoclone AK-Calibrant as per SSC guidelines were used.
Results: Normal reference ranges were established in 50 normal healthy donors (see Table 1). Excellent between-day imprecision was obtained for all 3 reagents and acceptable on-board stability was observed. Good agreement was obtained between methods in 130 samples from patients receiving warfarin. The FII sensitivity of NewPT was similar to reagents A and B but NewPT was more sensitive to FV, FVII and FX. NewPT had similar sensitivity to reagent B for coagulation defects in liver disease and improved sensitivity compared to reagent A. No heparin interference was observed in plasma spiked with up to 1.5 IU/mL unfractionated heparin or low molecular weight heparin in NewPT or reagent A both of which contain a heparin-neutralising compound. The lupus anticoagulant sensitivity of all three reagents was similar. NewPT demonstrated dose responsiveness to Dabigatran, Apixaban and Rivaroxaban with steeper response curves than Reagent A or B.

Table 1

	NewPT	PT reagent A	PT reagent B
Normal range (s) $n = 50$	9.87–12.66	9.70–11.73	11.33–13.75
ISI	1.00	0.97	1.07
Between day imprecision %CV. Normal QC; Abnormal QC	0.81; 0.83	0.78; 1.55	1.30; 1.24
4 day on-board stability (%day 0)	-2.1 to -0.8	-4.8 to -0.8	-3.9 to 0.7
Mean INR (Warfarin $n = 130$)	3.06	3.02	3.09
Correlation vs. regent A (r)	0.92	–	0.94
Correlation vs. reagent B (r)	0.93	0.94	–
Mean INR in LA plasmas ($n = 53$)	1.87	1.87	1.62

Conclusions: NewPT showed comparable or improved performance relative to two widely used PT reagents and is suitable for use in the control of warfarin, detection of inherited factor II, V, VII and X deficiency and assessment of coagulopathy in liver disease.

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The control of heparin treatment with global hemostasis assays: a sensitivity analysis

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Background: Heparin therapy is accompanied with bleedings and thrombotic complication due to individual response. Dosage control by the standard assays poorly reflects the effect of the therapy and does not correlate with a clinical outcome. So an assay with higher sensitivity to a hemostasis prothrombotic changes and a heparin effect is needed.
Aims: The sensitivity of laboratory assays to the hemostasis state before and after unfractioned heparin (UFH) and low molecular weight heparin (LMWH) therapy was compared.
Methods: A receiver operating characteristic (ROC) curve analysis was used to evaluate changes in activated partial thromboplastin time (aPTT), thrombin generation test (TGT), thromboelastography (TEG) and thrombodynamics (TD) before and after UFH or LMWH injection in prophylactic (124 patients after surgery and 53 patients with hemoblastosis) and therapeutic (124 patients with deep vein thrombosis) doses. Blood was sampled before injection, at the time of maximal heparin concentration in blood and before the next heparin injection.
Results: Hypercoagulation before heparin treatment was detected by TGT, TEG and TD but not by aPTT. The area under ROC curve (AUC) was maximal for TD (0.88 ± 0.08 for the clot growth rate), intermediate for TGT (0.75 ± 0.15 for the maximal amplitude) and TEG (0.76 ± 0.17 for the angle alpha) and minimal for aPTT (0.70 ± 0.05 for UFH and 0.60 ± 0.09 for LMWH).
Conclusions: Global hemostasis assays TD, TGT and TEG showed better sensitivity than aPTT to prothrombotic hemostasis changes before anticoagulant therapy and to heparin effect after both prophylactic and therapeutic treatment of UFH and LMWH.

CA40

Evaluation of laboratoricaly monitored efficiency of new oral anticoagulants rivaroksaban and dabigatran

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Background: Rivaroxaban and dabigatran are new oral anticoagulants that specifically inhibit one coagulation factor, factor Xa and thrombin. Both compounds have stable pharmacologic profiles, making regular monitoring as required for vitamin K antagonists, unnecessary. However, in specific circumstances, such as in renal insufficiency, bleeding, urgent surgical procedures, patients using these anticoagulant drugs may need laboratory monitoring and perhaps dose adjustments.
Aims: We want to show the verification process of introducing the anti-Xa test for Rivaroxaban and diluted trombin time (dTT) Hemoclot for Dabigatran on the analyzer BCS XP (Siemens) in our laboratory.
Methods: In the verification process, we evaluated three different parameters: repeatability of test anti-Xa for rivaroxaban, the concentration of anti Xa and diluted trombin time (TT) hemoclot fot dabigatran in healthy blood donors, and assessment of drug efficiency in plasma by measuring its concentration 2–4 h after ingestion in patients treated with the new anticoagulants (Rivaroxaban and Dabigatran).
Results: To assess the reproducibility of the test anti-Xa assay, two levels of commercial controls -Technoview Rivaroxaban Control Low and Medium were used.We calculated the CV (%) after 10 measurements Technoview Rivaroxaban Control Low and Medium which was at a low control of 2.41% and in the medium - high control of 3.24%. Concentration values of Rivaroxaban in the normal population ($N = 36$) were 1,29 ± 1,6 μg/L, and < 20 μg/L for Dabigatran (N = 10).
Conclusions: According to the results, manufacturer's recommended values for both tests suggesting that there is no drug effect (anti-Xa < 5 μg/L and hemoclot < 30 μg/L) were confirmed. We have also found that the concentration range of the drug 3 h after ingestion is wide for both test (anti-Xa from 74–161 μg/L and the 60–270 μg/L for dTT), since the final concentration depends primarily on the renal function of the patient.

CA41

Measuring direct oral anticoagulants in standardized fully automated thrombin generation on ceveron® alpha

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Background: The increasing use of the DOACs creates the need of their measurement in clinical routine. A modified thrombin time-based assay can be used for the measurement of thrombin inhibitors and the direct Xa inhibitors can be measured with a chromogenic anti-Xa assay. However, these assays only measure the initiation phase of the coagulation cascade.
Aims: In the present wanted to see if the thrombin generation assay (TGA), which measures the entire thrombin generation process, could be used to better discriminate the inhibitory profile of the DOACs in patients.
Methods: For this proof of concept study, platelet-poor plasma spiked with Apixaban, Rivaroxaban, Dabigatran, Arixtra or LMWH as well as Phenprocoumon plasma with different INR values was tested in the TGA. As initiators of thrombin generation two triggers differing in phospholipid concentrations were used. Analysis was performed on the coagulation analyzer Ceveron® alpha TGA.
Results: All anticoagulants, except Dabigatran, which only shows a response at higher doses, inhibited thrombin generation in a concentration-dependent manner after activation with both triggers, influencing all TGA parameters. Percent inhibition was calculated for Peak Thrombin (Peak) and Area under the Curve (AUC) values.
For the Xa inhibitors Rivaroxaban and Apixaban the inhibition of Peak values was more pronounced as for AUC, showing a plateau for both parameters at DOAC concentrations above 200 ng/mL, were as for high heparin concentrations and INR values at 4.5 both parameters showed almost complete inhibition. Dabigatran showed a 6 fold prolongation of the Lag time, inhibition of Peak and AUC was below that for Xa inhibitors.
Conclusions: Differences in TGA parameters between the anticoagulants reflect their differing inhibiting capacity in human plasma. Thrombin generation measurement in samples with DOACs at clinically relevant plasma levels could therefore serve as a fine-tuned indicator for hemostatic balance (individualized therapy) in patients using the DOACs.

CA42

Distinct effects of apixaban and rivaroxaban on thrombin generation and coagulation during recovery after total hip arthroplasty

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Background: Factor Xa-inhibitors (FXaI) apixaban (Apix) and rivaroxaban (Riva) are used in thrombophylaxis after major elective orthopedic surgery. Only a few real-life studies use patient samples in measuring coagulation activity.
Aims: To examine the FXaI effects on thrombin generation (TG) and coagulation in patients after total hip arthroplasty (THA) during 1 month follow-up.
Methods: Study group consisted of 20 unilateral THA patients with prophylactic postoperative Riva (10 mg od) and 22 with Apix (2.5 mg bid). We collected blood samples before and 3 h after drug intake at 4 time points: preop, postop day 1, week 1 (day 2–8) and day 28. APTT, PT, albumin, Hgb, CRP and leukocytes were immediately analyzed. Calibrated anti-FXa activity, and TG (Calibrated Automated Thrombogram) assays were performed from frozen samples.
Results: Hgb and albumin decreased reaching minimum at week 1, 105 g/L (range 78–132 g/L) and 29 g/L (range 24–37 g/L), respectively, while CRP and leukocytes increased. APTT and PT correlated poorly with Riva (APTT R^2=0.07, PT R^2=0.44) and APTT with Apix (R^2=0.07), remaining mainly within the reference interval. Mean Apix concentration varied 8-fold, 19–153 ng/mL at peak levels, whereas Riva only 1.5-fold, 111–183 ng/mL. Both FXaIs prolonged TG lag time at all time points ($P < 0.001$). Riva decreased ETP at all time points, reaching minimum at day 28 (536 nM/min at Riva 184 ng/mL, $P < 0.001$). Yet, with Apix, ETP did not change until at day 28 (990 nM/min at Apix 112 ng/mL, $P = 0.01$).
Conclusions: Expectedly, PT and APTT lacked sensitivity to Apix and Riva. Riva attenuated TG outright, but ETP did not decrease in response to Apix until day 28. The highly inflammatory and thrombogenic state of the THA patients influences TG, possibly contributing to the differences between FXaIs.

CA43

Performance of a new chromogenic apixaban assay on automated analyzers

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Background: Apixaban (ELIQUIS) is an oral direct factor Xa inhibitor. Although Apixaban does not require regular monitoring, its assessment in certain situations is helpful.
Aims: Performance of the HemosIL Apixaban Assay (Instrumentation Laboratory, Bedford, MA, USA) to measure Apixaban concentrations in human citrated plasma was evaluated on the ACL TOP Family analyzers.
Methods: The HemosIL Apixaban Assay is an anti-Xa chromogenic assay using IL's HemosIL Liquid Anti-Xa, Apixaban Calibrators and Apixaban Controls kits. A five-point calibration curve was automatically prepared on the ACL TOP using two Apixaban Calibrators (0 and ~500 ng/mL) and the assay is quality controlled by two levels of controls (~75 and 300 ng/mL). The precision, LoD, linearity, interference and method comparison were evaluated. Open-vial on-board and closed-vial stabilities (2–8°C and −20°C) were evaluated for the reconstituted Calibrators and Controls.
Results: The precision CV was ≤ 5% with Apixaban samples recovering within their established limits. The test had a LoD of 6 ng/mL and was linear between 15 and 1000 ng/mL with reflexive testing. The assay was not interfered by hemoglobin (up to 300 mg/dL), bilirubin (up to 25 mg/dL) and triglycerides (up to 1156 mg/dL). The method comparison outcomes were as follows:
a)internal studies: vs Biophen DiXaI: $R = 0.996$ and slope = 1.014; vs LC-MS/MS: $R = 0.982$ and slope = 1.077;
b)an external performance evaluation vs Biophen DiXaI: $R = 0.997$ and slope = 1.06. The reconstituted Calibrators and Controls were stable for 8 h on-board, 7 days at 2–8°C and 60 days at -20°C with a single freeze/thaw.
Conclusions: IL's HemosIL Apixaban Assay on ACL TOP is reliable and accurate for measuring Apixaban concentrations in human citrated plasma. It has good sensitivity in the low range to verify clearance of Apixaban prior to surgery, as well as sensitivity in the clinical and high ranges. The test shows good component stability, sample precision and comparability to predicate devices.

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Safety and efficacy of dabigatran compared with warfarin in patients with acute venous thromboembolism enrolled in RE-COVER®/RE-COVER II™ in Western Europe

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Background: The efficacy and safety of dabigatran etexilate (DE) compared with warfarin (W) for the treatment of acute venous thromboembolism (VTE) was investigated in the RE-COVER®/RE-COVER II™ trials.
Aims: To compare the safety and efficacy of DE vs. W in the Western European sub-population using pooled RE-COVER®/RE-COVER II™ data.
Methods: Patients with acute VTE on parenteral anticoagulation received W (international normalized ratio 2–3) or DE (150 mg twice daily) for 6 months, with a 30-day follow up. The primary efficacy outcome was recurrent, symptomatic VTE/VTE-related death. Safety outcomes were major bleeding events (MBEs), a composite of MBEs or clinically relevant non-major bleeding events (CRNMBEs) and any bleeding during the treatment phase. Outcomes were centrally adjudicated. Western European sub-population data were analysed using a Cox regression model with factor treatment stratified by study, assuming different baseline hazards per study.
Results: This sub-analysis included 1239 patients for the efficacy analysis (DE $n = 613$; W $n = 626$) and 1192 patients for safety (DE $n = 588$; W $n = 604$) from all 13 Western European countries participating in RE-COVER®/RE-COVER II™. The rate of VTE/VTE-related death was 2.1% ($n = 13$) for DE vs. 2.9% (n = 18) for W, which was not statistically significant (hazard ratio [HR] 0.74; 95% confidence interval [CI], 0.36–1.5). Rates of MBEs were similar between treatment arms (1.4% for DE [$n = 8$] and 1.3% for W [$n = 8$]; HR 1.02; 95% CI, 0.38–2.71). Rates of MBEs/CRNMBEs were significantly lower with DE (5.1%; $n = 30$) than with W (9.4%; $n = 57$) (HR 0.52; 95% CI, 0.34–0.82). Any bleeding events were statistically lower with DE (17.5%; $n = 103$) than W (23.8%; n = 144) (HR 0.7; 95% CI, 0.54–0.90).
Conclusions: In Western European countries, no difference regarding efficacy was seen between the DE and W arms. MBE/CRNMBE and any bleeding events were significantly reduced with DE compared with W. Western European data do not differ from results in the rest of the world.

CA45

Efficacy and safety of dabigatran vs. warfarin for the treatment of acute venous thromboembolism: a pooled analysis of 90-day data from RE-COVER™ and RE-COVER™ II

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Background: Anticoagulant treatment for 90 days is recommended in patients with deep vein thrombosis (DVT) or pulmonary embolism (PE) provoked by transient venous thromboembolism (VTE) risk factors and in certain patients with unprovoked DVT or PE.
Aims: To compare the efficacy and safety of dabigatran etexilate (DE) with warfarin (W), when given for the recommended 90 days, in patients with acute VTE (PE/symptomatic proximal DVT) in the overall population and in patients with one transient VTE risk factor using pooled RE-COVER™/RE-COVER™ II trial data.
Methods: Patients received W or placebo plus parenteral anticoagulation for ≥ 5 days until the international normalized ratio (INR) was ≥ 2 at two consecutive measurements. Parenteral therapy was discontinued and patients continued W (INR range 2.0–3.0) or received DE 150 mg twice daily for 6 months (double-dummy phase).
Results: For the overall population up to Day 90, fewer patients on DE vs. W had recurrent VTE/VTE-related deaths (counted from the start of parenteral therapy); however, the difference was not significant (see Table). The incidence of major bleeding events (MBEs) and MBEs/clinically relevant non-major bleeding events (CRNMBEs) (counted from the start of the double-dummy period) was lower with DE vs. W; the difference was significant for MBE/CRNMBEs. There

were 1202 patients with one transient VTE risk factor. Up to Day 90, recurrent VTE/VTE-related death occurred in 1.7% patients on DE and 2.1% on W (hazard ratio [HR], 0.82; 95% confidence interval [CI] 0.36, 1.86). MBEs occurred in 0.3% of patients on DE and 0.6% on W (HR, 0.60; 95% CI 0.10, 3.59). MBEs/CRNMBEs occurred in 2.0% of patients on DE and 4.1% on W (HR, 0.48; 95% CI 0.24, 0.97).

Table

	VTE/VTE-related deaths*		MBEs†		MBE/CRNMBEs†	
	DE	W	DE	W	DE	W
Overall population						
Patients, n/N (%)	47/2553 (1.8)	52/2554 (2.0)	17/2456 (0.7)	28/2462 (1.1)	65/2456 (2.6)	146/2462 (5.9)
DE vs W: HR (95% CI)‡	0.90 (0.61, 1.34)		0.60 (0.33, 1.10)		0.44 (0.33, 0.59)	
Subgroup with one transient VTE risk factor						
Patients, n/N (%)	11/631 (1.7)	12/571 (2.1)	2/612 (0.3)	3/543 (0.6)	12/612 (2.0)	22/543 (4.1)
DE vs W: HR (95% CI)‡	0.82 (0.36, 1.86)		0.60 (0.10, 3.59)		0.48 (0.24, 0.97)	

*Dataset for the efficacy analysis comprised 2553 patients on dabigatran and 2554 patients on warfarin.

†Dataset for the safety analysis comprised 2456 patients on dabigatran and 2462 patients on warfarin.

‡Cox Model assuming different baseline hazards for the studies and a common treatment effect.

Conclusions: DE showed similar efficacy and a lower risk of MBE/CRNMBEs vs. W in the overall population and in those with one transient VTE risk factor.

CA46

Application of basic and special coagulation tests for measuring rivaroxaban activity

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Background: Rivaroxaban (Xarelto®) is an oral anticoagulant, direct and selective factor Xa inhibitor, which has a predictable pharmacokinetic. Coagulation studies will be needed in cases of hemorrhagic or thrombotic complication, urgent or planned surgery, and certain specific situations (extreme weight, renal insuficiecia, drug interactions).
Aims: The aim of this study is to assess the biological effect of rivaroxaban in a group of patients, and analyze the correlation between two techniques.
Methods: Multicenter study included 51 adult patients with VTE and AF treated with rivaroxaban. Exclusion criteria: patients taking rivaroxaban as primary prophylaxis in orthopedic surgery. Data collected: age, sex, weight, indication, thrombotic and hemorrhagic history, creatinine, glomerular filtration rate, concomitant diseases, concomitant medication, bleeding risk factors, prothrombin time, cephalin time, fibrinogen. Anti-Xa activity were determined using HemosIL Liquid Heparin kit. Concentration of rivaroxaban was tested by a chromogenic assay using specific calibrators TECHNOVIEW Rivaroxaban High Set Cal. Determinations were performed at 2 and 24 h after ingestion.
Results: The mean age was 65.37 ± 16.37 years, 45% vs 55% (women vs men). 18% received a dose of 15 mg/24 h, 82% received 20 mg/24 h. The average weight was 73.0 ± 11.7 kg. Figures 2 and 3 show the correlation between levels of rivaroxaban and anti-Xa activity and PT. Mean concentration was 239.70 and 29.2 ng/ml at 2 and 24 h after dosing. There is an interindividual variability in the concentration of rivaroxaban in patients older (289.5 ng/ml) and under 80 (225 ng/ml); which coincides with lower glomerular filtration rate (59.9 vs 78/1 ml/min).
Conclusions: Anti Xa chromogenic assay with calibrators for rivaroxaban is a quantitative method, easy to perform, allowing us to determine its plasma level. Applying a correction factor rivaroxaban plasma concentration (ng/ml) can be estimated from anti-Xa activity calibrated for heparin (IU/ml) test.

CA47

EffectiVeness and safety of rivaroxaban therapy in daily-care patients with unusual venous thromboembolism - results of the prospective dresden NOAC registry (NCT01588119)

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Background: Rivaroxaban is approved and widely used to treat deep vein thrombosis but it is unclear, if rivaroxaban can also be safely used to treat venous thromboembolism (VTE) in unusual sites such as cerebral (CVT) or splanchnic vein thrombosis (SVT).
Aims: To evaluate the effectiveness and safety of rivaroxaban in unusual site VTE.
Methods: Subgroup analysis of unusual site VTE patients from the prospective, non-interventional Dresden NOAC registry.
Results: Until December 31th 2015, 21 patients received rivaroxaban for treatment of VTE in unusual sites (47.6% female, mean age 55 years; 71.4% idiopathic) which consisted of 16 (76.2%) SVT, 4 (19.0%) CVT and 1 (4.8%) ovaric vein thrombosis. Median time between VTE diagnosis and initiation of rivaroxaban was 32 day (25th/75th percentile 14; 170 day).
Mean exposure time of rivaroxaban was 265 ± 205 days. During treatment, no recurrent VTE was observed. 10 patients had a total of 16 bleeding complications which consisted of 8 ISTH minor bleeding, 6 clinically relevant non-major bleeding and 2 major bleeding (both with drop of haemoglobin during acute gastrointestinal bleeding). Bleeding sites consisted of gastrointestinal tract (6), menorrhagia (5), hematoma (2), epistaxis (1), gingival bleeding (1), haemoptysis (1). Bleeding led to rivaroxaban discontinuation in one case with major gastrointestinal bleeding.
During follow-up (mean 13.4 months; range 0.5–48.6), rivaroxaban was discontinued unscheduled in two (9.5%) patients. Six patients (28.6%) had a scheduled end of treatment. No patient died during follow-up.
Conclusions: In daily care, treatment of unusual site VTE with rivaroxaban seems common. Since no anticoagulant is specifically approved for these conditions, rivaroxaban may be regarded as an alternative, since it seems effective and acceptably safe. However, bleeding complications were frequent and predominantly occurred as gastrointestinal or abnormal uterine bleeding. Overall, persistence with rivaroxaban therapy was high.

CA48

Reasons and consequences of vitamin K antagonists (VKA) discontinuation in very elderly patients with non valvular atrial fibrillation (NVAF). an inception cohort study

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Background: Non-valvular atrial fibrillation (NVAF) is very common in elderly. Oral anticoagulant therapy with vitamin K antagonists (VKA) such as warfarin is greatly effective in stroke prevention but may also expose to bleedings, which are more frequent in the elder and frail patients. VKA discontinuation rates in NVAF patients are reported to be high, especially in elderly.
Aims: To report the reasons for VKA discontinuation and the clinical outcomes in a cohort of very elderly patients with NVAF attending a large size anticoagulation clinic.
Methods: This is a retrospective analysis of an inception cohort study (VENPAF, i.e. Very Elderly NVAF patients naïve to VKA therapy). Characteristic of patients who discontinued warfarin were collected, included the person responsible for suspension and the reason produced for stopping the treatment. We then analysed clinical factors associated to VKA discontinuation and finally analysed mortality, bleedings and ischemic events before and after suspensio.
Results: Discontinuation rate was 18.5%. Warfarin suspension was mainly decided by the specialist during hospitalization, because of perceived frailty/low life expectancy or previous bleeding. Independent factors associated to discontinuation are displayed in the Figure. During VKA treatment, patients who discontinued warfarin showed higher ischemic and bleeding complications and lower time in therapeutic range (TTR) as compared to patients who persisted in warfarin. Even after warfarin discontinuation ischemic and bleeding complications remained high (from 3.14 to 3.49 %pt/y for thrombo-embolic events and from 10.8 to 3.5 %pt/y for major bleedings).
Conclusions: Anticoagulation management in very elderly patients with NVAF can be problematic. Discontinuation rate is very high and patients who discontinue treatment are at very high risk of complication irrespective of anticoagulation. Current antithrombotic treatment in this class of very elderly, fragile and complex patients, is still a challenge.

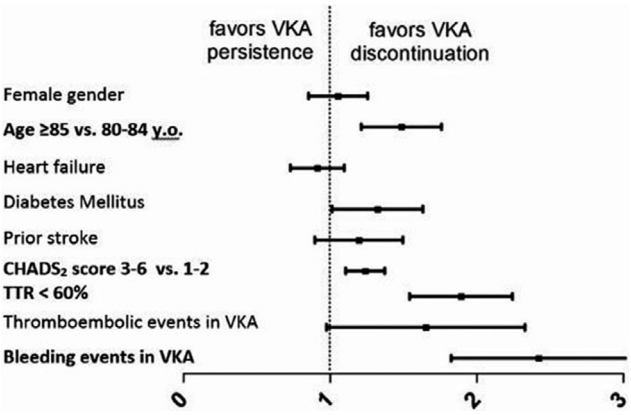

Figure Clinical factors associated with discontinuation.

CA49

The frequency of gene polymorphism among patients with hemorrhagic complications connected with warfarin therapy

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Background: Warfarin is the indirect anticoagulant most commonly prescribed to patients with high risk of thromboembolic complication. Polymorphisms of VCORC1 G1639A and cytochrome CYP2C9 genes influence the efficiency of anticoagulant therapy.
Aims: The comparison of results of genetic research and laboratory data results is made in order to study correlation between patients with hemorrhagic complications connected with Warfarin therapy.
Methods: The study includes 39 patients (19 men and 20 women) with the average age of 71.5 ± 8.6 years. Laboratory examination included INR determination, genetic typing of gene VCORC1 G1639A polymorphisms, cytochrome P450 CYP2C9 (*1,*2,*3) enzyme.
Results: During the study it was found out that the genotype G1639G tends to be least common ($p<0.09$), only 23%. Polymorphism A1639A is more common and comes to 35.9%. This number appeared to be higher than in Caucasian population in general. The average warfarin dose among patients with G1639G was higher (6.9 ± 2.9 mg) among patients with polymorphism A1639A it was 3.8 ± 2.3 mg. The frequency of polymorphism *1/*1 in CYP2C9 gene among target group was higher (69.2%; $p<0.01$) which corresponds to general population index among Caucasian. According to literature data, the frequency of gene type *1/*1 in CYP2C9 gene is 64.8%, frequency of gene types *1/*2* and 1/*3 is 30.6%. The average dose of warfarin among patients with polymorphism *1/*1 in CYP2C9 gene was 5.8 ± 3.1 mg, among patients with gene type *1/*2 - 3.3 ± 1.3 mg, with gene type *1/*3 - 3.5 ± 1.2 mg.
Conclusions: The polymorphism A1639A VCORC1 gene among patients with hemorrhagic complications during warfarin therapy appeared to be higher that among Caucasian population.

CA50

Prescribing errors with new oral anticoagulants at a regional base hospital in NSW, Australia

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Background: New Oral Anticoagulants (NOACs) are used as an alternative to Warfarin for prevention and treatment of stroke, pulmonary embolism and deep vein thrombosis. Paper based medication charts are used by the NSW health system, containing specific sections to chart VTE prophylaxis, or warfarin, but not other anticoagulants.
Aims: Identify errors in prescribing VTE prophylaxis or transitions to Heparin/LMWH for patients prescribed a NOAC. Identify any adverse events from these errors.
Methods: Hospital pharmacy provided a list of patients dispensed either Apixaban or Rivaroxaban over 12 months. Medication charts and progress notes were reviewed for prescribing errors and associated complications. Reason for admission, NOAC indication, and changes to anticoagulation medications were noted. Simple statistical analysis was performed.
Results: 250 patients were dispensed either Apixaban (N = 82) or Rivaroxaban (N = 168) between May 2014 to May 2015. 92 were analysed as a sample (Apixaban 38, Rivaroxaban 54). Reasons for admission included Atrial Fibrillation or other arrhythmia (37%), General Medical (33%) and Embolic events (11%). Indications for NOAC use were Atrial Fibrillation (83%), PE (11%), DVT (4%), DVT/AF & PE (2%). 33 patients were switched from NOAC to therapeutic Heparin/LMWH during admission. 4 of these were erroneously given both NOAC and therapeutic Heparin/LMWH. 12 patients were

concurrently charted NOAC and VTE prophylaxis, with 4 given concurrent doses. There were no complications as a result of the prescribing errors. 19.5% of medication charts reviewed had errors.
Conclusions: We have found evidence that the introduction of NOACs into real world practice creates a risk of medication errors with potential clinical risk to patients. The introduction of further NOACs (edoxaban, betrixaban etc) is likely to increase this risk. Hospitals should take measures to minimise the potential risks from the increasing complexity of agents available.

CA51

Comparison of locally measured activated partial thromboplastin time (aPTT) to central laboratory aPTT when measuring reversal of dabigatran using idarucizumab

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Background: RE-VERSE AD is an ongoing open label study evaluating reversal of dabigatran anticoagulation in emergency settings using the specific reversal agent idarucizumab (ida). Group A (GrA) experienced uncontrolled or life-threatening bleeding and Group B (GrB) required an urgent invasive procedure. Participating sites collected samples for local measures of anticoagulant activity and sent samples for central measurement.
Aims: This analysis compares results of local aPTT to centrally measured aPTT and diluted thrombin time (dTT).
Methods: The study was approved by local ethics committees and written informed consent was obtained. Local aPTT was assayed at baseline, after the 1st 2.5 g ida, 30 min after the 2nd 2.5 g and 12 hrs later, and were compared to central lab aPTT at similar time points. Values for local aPTT were normalized to allow comparison across sites and with centrally measured values.
Results: In the first 90 patients enrolled, 34/51 in GrA and 29/39 in GrB had aPTT baseline values above local upper limit of normal (ULN) and were evaluable for reversal. In central lab data, 39/51 in GrA and 26/39 in GrB had elevated aPTT. Baseline anticoagulation was variable due to different dabigatran levels in patients upon study entry. After ida dosing, 100% reversal was achieved in 74% patients in GrA and 66% in GrB based on local aPTT as compared to 95% and 85% based on central lab aPTT. Normalized local aPTT results correlated with central aPTT (R^2= 0.8304). Central and local aPTT correlated to the primary outcome of reversal as dTT, 100% reversal was achieved in 98% of GrA and 93% of GrB patients after ida infusion.

Table Local and central lab aPTT and dTT (mean±SD,s).

	Baseline	Between vials	10–30 min	12 h
Local	55 ± 34	35 ± 24	33 ± 19	33 ± 16
Central	65 ± 59	36 ± 25	36 ± 22	36 ± 16
dTT	54 ± 31	31 ± 14	30 ± 8	33 ± 16

ULN (s): 35.1 normalized local aPTT, 39.8 central aPTT, 35.5 dTT

Conclusions: These data show that locally measured aPTT is comparable to centrally measured aPTT, and consistent with measuring reversal of dabigatran anticoagulation using dTT. Thus aPTT may also provide semi-quantitative guidance regarding the intensity of dabigatran-associated anticoagulation and its reversal in clinical settings.

CA52

Warfarin as a new oral anticoagulant agent?

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Background: Monitoring warfarin with Fiix-prothrombin time (Fiix-PT) that is only affected by coagulation factors II and X, stabilizes anticoagulation and reduces thromboembolism compared to PT/INR monitoring.
Aims: We aimed to compare outcome in non-valvular atrial fibrillation (NVAF) patients treated with Fiix-warfarin, direct oral anticoagulants (DOACs) or PT-warfarin.
Methods: Systematic efficacy and safety assessment of Fiix-warfarin, PT-warfarin and DOACs in NVAF patients by retrieving data from the Fiix-trial and the four major phase III DOAC trials. Pre-specified outcomes included stroke and systemic embolism (SSE), SSE and myocardial infarction (MI), major bleeding (MB), composite major vascular events (SSEMI and MB; CMVE), and deaths. We calculated relative risk and 95% CI and 95% confidence limits (CL) for each outcome and did meta-analysis using fixed- and random effects modelling.
Results: There were 613 and 628 observation years with Fiix-warfarin and PT-warfarin in the Fiix-trial and 70,628 and 57,962 with DOACs and PT-warfarin in DOAC trials. Trial populations were comparable although death rates were lower in the Fiix-trial. Compared to pooled PT-warfarin, Fiix-warfarin reduced SSE (RR 0.54;95% CI 0.26–1.10/ 95% CL< 1.00), SSEMI (0.51;0.26–0.99/< 0.90), MB (RR 0.63;0.37–1.07/< 0.99) and CMVE (RR 0.66;0.43–1.00/< 0.94). Vascular death was lower (RR 0.13;0.04–0.47/< 0.42). Compared to pooled DOACs, Fiix-warfarin consistently had lower point-estimates for the RR for efficacy and safety but only significant for lower death rates (vascular death RR 0.14;0.04–0.49/< 0.43). Meta-analysis comparing Fiix-warfarin and DOACs to PT-warfarin, consistently found Fiix-warfarin to have the lowest point estimates for efficacy.

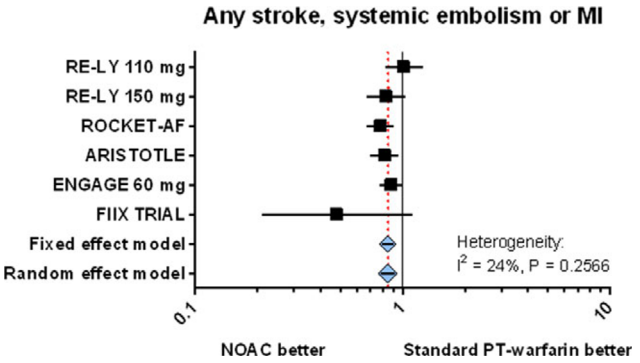

Figure Stroke, systemic embolism and MI.

Conclusions: Fiix-warfarin reduces risk of vascular events in NVAF patients compared to PT-warfarin as much as do DOACs. Fiix-warfarin is an improved oral anticoagulant compared to standard PT-warfarin.

CA53

Comparing mechanisms of action of novel anticoagulant reversal agents through studies on binding partners and on clot formation and clot structure

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Background: Anticoagulants (ACs) used to prevent blood clotting and to treat thromboembolic events include unfractionated heparin (UFH), low molecular weight heparins (LMWHs), fondaparinux (fonda), and oral inhibitors (NOACs) of thrombin and fXa. Bleeding risks associated with these ACs demand careful monitoring and neutralization with a suitable antidote (AD). The UFH AD protamine has many known limitations. Improved ADs in development include: UHRA7 a universal heparin AD; Andexanet alfa (AnXa), an AD for NOACs, UFH, LMWHs, and fonda; and, N1,N1-[piperazine-1,4-diyl-bis(propane-1,3-diyl)]-bis-L-argininamide (PER977), a synthetic reported to reverse UFH, LMWH and certain NOACs. However, the binding partners to each of these ADs and their role in the associated mechanism(s) of action have not been fully defined or compared.

Aims: To determine

1) the ACs and coagulation-pathway components to which each AD has binding affinity,
2) the neutralization activity of each AD against edoxaban (edox) and enoxaparin (enox), and
3) the influence of each AD on clot formation/morphology in the absence/presence of AC.

Methods: Binding constants are measured by isothermal titration calorimetry (ITC); clot formation kinetics and strengths before and after neutralization by thromboelastography (TEG) and coagulation assays (CAs); and structures of fibrin clots and blood clots before/after neutralization by SEM.

Results: ITC confirms binding of: UHRA7 (< μM K_d) to UFH, enox and fonda, but not to edox; AnXa to edox (nM K_d) and ATIII-complexed with enox or fonda (< μM K_d); and PER977 to enox (μM K_d), but not to edox or fonda. A $K_d \leq$ μM correlates with neutralization activity determined by TEG or CAs, except for the PER977/enox system, where no reversal activity was observed. Standard metrics of clot structure correlate only weakly with neutralization activity.

Conclusions: This is the 1st study to compare three AC reversal agents under development, each of which appears to exert its reversal activity through a unique mechanism of action.

CA54

Matrix effect of commercially available direct oral anticoagulant standards: a look through the limits and the need for standardization of current calibrators

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Background: Several laboratories still use routine coagulation tests, such as the prothrombin time (PT) and the activated partial thromboplastin time (aPTT) to estimate the intensity of anticoagulation in patients treated by the direct oral anticoagulants (DOACs). In addition, it is mandatory that all laboratories are aware about the sensitivity of their own reagent/coagulometer combination.

Aims: To assess the variability of baseline value, sensitivity and robustness of several coagulation tests using commercially available and home-made set of DOAC calibrators.

Methods: PT, aPTT, Thrombin Time (TT), Ecarin Clotting Time (ECT), Ecarin Chromogenic Assay (ECA), diluted TT (dTT), chromogenic anti-Xa assay, diluted Russell Viper Venom Time (dRVVT) and the thrombin generation test (TGT) have been performed on two commercially available (from Hyphen BioMed and Diagnostica Stago) and one home-made sets of dabigatran, rivaroxaban and apixaban calibrators. Homemade calibrators were realized from a normal pooled plasma spiked with DOACs from 0 to 500 ng/mL. Several reagents have been used for each tests which were carried out on a STA-R Evolution coagulometer.

Results: All clotting tests sensitive to factor deficiencies, i.e. PT, aPTT, TT, ECT and dRVVT, show calibrator-dependent dose-response curves. This effect is not observed in tests where the contribution of the components of the plasma sample is negligible. This includes ECA and chromogenic anti-Xa assays which are normalized with prothrombin and factor Xa, respectively. Interestingly, blank samples from manufacturers' calibrators show abnormal results for several parameters of the TGT.

Conclusions: Laboratories should not estimate the sensitivity of their coagulation tests using commercially available calibrators to avoid misleading results. In addition, it is of upmost importance to consider and work on the establishment of international standards for the manufacturing of DOACs′ calibrators.

CA55

Concentration dependent effects of edoxaban on thrombin generation kinetics and physical clot characteristics by TEG 6S and CAT assay

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Background: Edoxaban is a recently approved factor Xa inhibitor. Concentration dependent effects of edoxaban on physical characteristics of clot and thrombin generation kinetics are not known.

Aims: To study the in vitro concentration dependent effects of edoxaban on physical characteristics of clot and thrombin generation kinetics.

Methods: Platelet-fibrin clot strength (P-FCS), and reaction time (R) in whole blood using the point-of-care TEG6s with the anticoagulant cartridge and endogenous thrombin potential (ETP), lag time, peak thrombin concentration (PTG), and time to peak thrombin concentration (ttpeak) in plasma using the calibrated automated thrombogram (CAT) assay were determined after 30 min of incubation with 0, 30 (subtherapeutic range), 300 (therapeutic range) and 900 nM (supratherapeutic range) edoxaban in citrated blood samples collected from healthy volunteers and patients with HF with and without hypercoagulability (defined as ≥ 65 mm P-FCS) (total $n = 43$).

Results: Overall, a concentration dependent effect of edoxaban on R, lag time (indicators of anticoagulant effect), PTG, and ttpeak (P for trend < 0.001 for all) was observed. The anticoagulant effect was more pronounced in subjects with normal coagulability (see Table).

Table

	R-Value (min):TEG6s Assay				Lag Time (min):CAT Assay			
	0 nM Edoxaban	30 nM Edoxaban	300 nM Edoxaban	900 nM Edoxaban	0 nM Edoxaban	30 nM Edoxaban	300 nM Edoxaban	900 nM Edoxaban
Overall (n=43)	0.6±0.2	1.2±0.3	2.3±0.3	3.1±0.6	4.4±1.2	5.4±1.6	6.9±1.6	9.1±2.6
HV-NC (n=12)	0.7±0.2	1.3±0.3	2.3±0.2	3.2±0.3	4.5±1.1	5.6±2.1	7.1±1.5	9.0±2.3
HV-HC (n=11)	0.5±0.1	1.1±0.3	2.1±0.2	2.7±0.4	3.9±0.7	5.0±1.0	6.0±1.2	8.8±2.0
HF-NC (n=10)	0.9±0.8	1.3±0.3	2.6±0.3	3.7±0.6	4.9±1.6	5.9±1.3	7.8±1.9	10.5±3.7
HF-HC (n=10)	0.7±0.4	1.2±0.3	2.2±0.4	2.5±0.8	4.0±1.0	4.5±1.3	6.5±1.6	7.9±1.9

HV= healthy volunteers, HF= heart failure patients, NC=normal coagulability, HC=hypercoagulability

Conclusions: The observed concentration dependent effects of edoxaban on reaction time, lag time, ttpeak, and PTG suggest that the TEG6s and CAT assays can be used to qualitatively and quantitatively determine anticoagulant effects of edoxaban and may facilitate personalized therapy.

CA56

Acute ischemic stroke after administration of kcentra for apixaban-*associated Intracranial Hemorrhage*

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Background: Novel non-Vitamin K Oral Anticoagulants are being increasingly used for stroke prevention in patients with nonvalvular atrial fibrillation and also for prevention and treatment of deep vein thrombosis (DVT) due to ease of dosing and simplicity of monitoring. Due to lack of a FDA-approved direct antidote and reversal agent for NOACs, Kcentra (prothrombin complex concentrates, human) have been suggested as a useful approach for NOACs reversal in the case of severe, life-threatening bleeding; however, a consensus has not been reached on its clinical benefit.
Aims: The procoagulant effect of Kcentra has raised concerns about increased risk of arterial thromboembolic events with its administration.
Methods: A 43 years old female with past medical history significant for Diabetes Mellitus, hypertension, chronic kidney disease on hemodialysis and DVT for which she was taking Apixaban 5 mg presented to the ER with numbness of left upper and lower extremities. She was found to have a large parieto-occipital ICH on her non-contrast CT scan. On admission INR/ PT values were 1.43/16.6. Initially, the decision was made to stop Apixaban and observe her closely in neuro- intensive care unit. Six hours later, her mental status deteriorates and repeat head CT showed expansion of ICH with midline shift. INR/PT also increased to 2.02/ 25.8 respectively. The decision was made to use Kcentra to reverse of the INR. After a detailed discussion about the risks and benefits of Kcentra, 50 IU/kg of Kcentra was administered intravenously. INR/PT values dropped to 1.91/ 20.8.
Results: Next day, however, she developed new onset right sided hemiparesis and her CT head showed a large middle cerebral artery infarction.
Conclusions: Potential benefits of reversing agent, Kcentra, should be weighed against the risk of thromboembolic events, especially in patients with history of such events.

CA57

Unusual site thrombosis - epidemiology and outcome

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Background: Unusual site thrombosis (UST) is uncommon with few literature to guide diagnostic workup and appropriate therapy.
Aims: To analyse the epidemiology and outcome of patients presenting with UST.
Methods: Retrospective analyses of patients presenting with venous thrombosis. Patients presenting with UST underwent complete workup for thrombophilia (homocysteine, proteins C,S, ATIII, MTHFR, factor V leiden mutation), immunological markers (LAC, dsDNA, anticardiolipin antibodies, anti β2-GPI), PNH clone by flow cytometry, JAK2 mutation and CT scan chest/abdomen.
Results: We studied1574 patients presenting with deep vein thrombosis (DVT) over 11 years, of which 155 patients(112 males), median age of 32 years (range 1–70) presented with UST. The sites involved were abdomen(98,63%), cortical veins(33,21%), upper limbs +/- neck (22,14%) and eyes(2,1.3%). Abdominal sites were mesenteric/portal venous system(53/98,54%), isolated inferior vena cava-IVC(30/98,30%), splenic vein(9/98, 9.2%) and renal vein(5/98,5%). A potential aetiology was identified in 109(70%) cases with acquired causes in 102 (66%) and 7 inherited (Tables 1 and 2). 1 case each of acute lymphoblastic leukaemia and acute myeloid leukaemia presented with cortical thrombus. Among the 43 cases with unknown etiology, 4 had a positive family history of clot or recurrent abortions (2 each). Of 16 smokers, 7 also had additional acquired and 2 had inherited causes. Lower limb DVT preceded or followed the UST in 13(none with IVC thrombus). Patients were followed for median of 3 years (3 months to 9 years). Two patients with recurrent episodes had suboptimal anticoagulation before coming to us. Median duration of anticoagulation was 2 years (6 months to 4 years). No patient developed post thrombotic sequelae. None died due to thrombus.

Table 1 Acquired causes of unusual site thrombosis.

Acquired defect	SITE of thrombus (number)
Infection/inflammation (includes 2 cases of ulcerative colitis)	Abdomen(13), Pulmonary artery (1), CSVT (5)
Antiphospholipid antibodies (one case associated with pregnancy)	Abdomen (8), CSVT (4), Eye(1), Upper limb and neck (2)
Chronic liver disease (Including one case of non cirrhotic portal fibrosis)	Abdomen (12)
Malignancies (other than JAK2/ MPN)	Abdomen (9), pulmonary artery (1), upper limb and neck (7), CSVT (7)
Homocysteinemia (no MTHFR mutation in this series)	Abdomen (4), upper limb (1)
JAK2/MPN	Abdomen (5)
PNH	Abdomen(2), CSVT (2)
Pregnancy	Abdomen (2), CSVT (3)
Thrombocytosis (one case associated with iron deficiency)	Abdomen (2), CSVT (1)

Table 2 Inherited defects and other infrequent acquired causes.

Rare causes(acquired and inherited)	Site of thrombus(number)
Cardiomyopathy	abdomen(1)- mesentric vein thrombosis
Gastroenteritis/ dehydration	CSVT (1)
Celiac disease	Abdomen (1)- mesentric vein thrombosis
Median arcuate ligament	Mesentric vein thrombosis and superior mesentric artery blockage (1)
Surgery (renal calculus)	Abdomen(1)
PICC line, Chronic kidney disease, Trauma (one case post seizure and two cases of Paget-Schroetter disease post excercise)	Upper limbs (5)
AT III deficiency	Abdomen(2)
Factor V leiden heterozygosity	CSVT (2), Abdomen (1)
Protein C deficiency	CSVT (1), Upper limb (1)

Conclusions: UST was identified in 9.8% cases presenting with thromboses. Unlike lower limb DVT, an identifiable cause, which would potentially influence outcome and duration of anticoagulant therapy, was present in most cases of UST.

CA58

A comparative study using four different clot detection methods for inr monitoring in patients on vitamin k antagonist treatment

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Background: Vitamin K antagonist (VKA) treatment requires routine monitoring using the international normalized ratio (INR). The use of point-of-care (POC) coagulometers can simplify the management of VKA patients. However, different ways of measuring the INR may vary in their results.

Aims: To assess the accuracy of POC in measuring the INR during VKA treatment.

Methods: Consecutive adult patients attending the Anticoagulation Clinic at Mater Dei Hospital for VKA monitoring in August-September 2015 were screened. We included 30 patients deemed eligible for POC monitoring according to the local protocol (at least 3 consecutive INRs within the therapeutic range 1.9–3.2, no severe comorbidities) and 30 random patients. The INR was tested using a POC device (CoaguChek XS Plus, Roche Diagnostics) for both capillary and venous blood samples, a photo-optical (Sysmex CS-2100 or CA-1500, Siemens) and a mechanical clot detection system (Thrombolyzer XRC, Behnk Elektronik), and the manual tilting-tube technique. All laboratory tests used the same lot of human recombinant thromboplastin (Dade Innovin, Siemens). This study was approved by the local Ethics Committee and all patients provided written informed consent.

Results: Sixty patients were enrolled. Mean (SD) age was 68.5 (11.5) years, 43% were males. The most common indications for VKA were atrial fibrillation (63%) and venous thromboembolism (27%). The current mean (SD) warfarin dose was 4.5 (2.1) mg.

Median INR using the POC on capillary blood samples was 2.6 (range 1.4–5.8). The capillary POC showed a strong positive correlation with the venous POC ($r = 0.99$), the photo-optical method ($r = 0.97$), the mechanical method ($r = 0.96$) and the manual technique ($r = 0.93$, all p values < 0.001). The percentage of results within 0.5 INR units were 100%, 88.3%, 96.7% and 96.7% for each comparison, respectively.

Conclusions: The results of our study confirmed the POC device as a valid alternative to laboratory INR in patients on VKA treatment.

CA59

Warfarin dose assessment every 4 weeks vs. every 6 to 8 weeks in patients with stable international normalized ratios: a retrospective study

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Background: Guidelines recommend that patients on warfarin have international normalized ratio (INR) monitoring every 4 weeks, which in selected cases can be extended up to 12 weeks. That was based on a study with surrogate endpoint.

Aims: We performed a retrospective cohort study in clinical practice to evaluate whether assessment of warfarin dosing every 6 to 8 weeks is as safe as assessment every 4 weeks.

Methods: Primary outcome was composite of major bleeding and thromboembolic events and secondary outcomes were time in therapeutic range (TTR) and number of patients with dose change(s).

Results: 150 patients were included in the 4-week group and 36 patients in the 6–8 week group. Baseline patient characteristics were similar in the two groups including gender (males, 102 (68%) vs. 27 (75%)), and the use of aspirin (55 (37%) vs. 16 (44%)), with the exception for age (median age of 71 vs. 66; $P = 0.05$). The median $CHADS_2$ score was 2 in both groups. Proportion of patients with mechanical valves, whether aortic (36% vs. 33%) or mitral (3.3% vs. 0%) was similar in the two groups. The median follow-up duration was significantly longer for the 4-week group compared with the 6–8 week group (15 months vs. 11 months; $P < 0.001$). Number of patients who had dose changes was not different between the two groups (19 (13%) vs. 4 (11%); $P = 0.89$). The TTR was significantly lower in the 4-week group than the 6–8 week group (89.3% vs. 94.8%; $P = 0.003$). Three in the 4-week group (2%) vs. none of the patients in the 6–8 week group had a major event (odds ratio 0.58; 95% confidence interval, 0.03–11.43). Two events were a CVA, and 1 was a major bleeding.

Conclusions: Our data suggest that assessment of warfarin dosing every 6 to 8 weeks seems to be as safe as assessment every 4 weeks. A phase 3 trial comparing clinical outcomes of testing every 4 weeks vs. longer duration (e.g. 6–12 weeks) would be needed before prolonged intervals for dose assessments can be recommended for clinical practice.

CA60

Development of an INR_{RIVA} on plasma samples of patients on rivaroxaban

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Background: Rivaroxaban is a factor Xa inhibitor that has been approved for use as an anticoagulant. It has predictable pharmacokinetics and a favorable risk profile, allowing it to be administered in fixed doses without routine laboratory monitoring. However, monitoring of rivaroxaban levels in selected situations, such as acute bleeding, remains worthwhile. Current methods are not widely available whereas the commonly used prothrombin time (PT) is sensitive to rivaroxaban levels. PTs are also used to monitor Warfarin therapy and have been standardized for different thromboplastin reagents through the use of the International Normalized Ratio (INR). INR measurements have only been standardized for Warfarin and not for Rivaroxaban. INRs for rivaroxaban have been developed *in vitro* by spiking plasma samples with rivaroxaban however *in vivo* measurements using blood samples from patients on rivaroxaban has not been previously reported.

Aims: To develop an INR for Rivaroxaban using plasma samples from patients.

Methods: We measured PTs in patients on rivaroxaban in order to develop International Sensitivity Index (ISI) values for five commonly used thromboplastin reagents. Blood samples from 29 patients treated with rivaroxaban were collected 3 h after ingestion (to measure peak levels) and analyzed to determine their PT. The resulting PTs were then compared to a WHO standard and an ISI was calculated for each reagent.

Results: The ISI's calculated were 1.27 for the Recombiplastin 2G, 0.82 for the PT HS Plus, 0.97 for the Neoplastin C1 Plus, 3.05 for the Innovin, and 2.00 for the Thromborel S. These ISI values can now be used to determine an INR for rivaroxaban when using the same reagents and analyzers.

Conclusions: This is the first time an ISI has been calculated for patients treated with rivaroxaban and allows for rivaroxaban specific INRs (INR_{RIVA}) to be developed for the monitoring of rivaroxaban treatment.

CA61

Length of anti-coagulation in splanchnic venous thrombosis

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Background: Anticoagulation therapy of SVT is a clinical challenge. Patients are at risk of developing certain complications and may experience recurrence. Anticoagulant therapy remains the cornerstone of treatment and should be started as soon as possible to prevent recurrence. Many patients are left untreated because the risks associated with anticoagulant therapy are calculated to exceed its benefits. However, the majority of patients receive anticoagulation with heterogeneous timing of initiation, drugs, and doses.
Aims: To observe the length of anti-coagulation in splanchnic venous thrombosis.
Methods: Retrospective, observational study of case charts of hospitalized patients diagnosed with SVT at Aga Khan Hospital Karachi during January to June 2015. Patients suffering SVT were identified by using ICD 9 coding. Details were obtained from electronic medical record system.
Results: SVT was found in 17 patients. Presenting compliant was abdominal pain in 9 patients. Anticoagulation was not started in 8 patients because of either risk of bleeding or chronic nature of portal vein thrombosis. 5 patients were started on Enoxaparin 60 mg twice daily and Warfarin (5–10 mg daily). Enoxaparin was stopped after achieving therapeutic INR and warfarin was continued. 4 patients were started only on warfarin (5–10 mg daily). Only 1 patient had bleeding due to warfarin after 20 days of initiation so it was stopped and he received enoxaparin for around 12 months. Out of other 8 patients only one had unstable INR (i.e. time in therapeutic range< 60%). Warfarin was continued for more than 4 months in these patients with median time 5.3 months. No episode of recurrence was reported in any of these patients till to date.
Conclusions: Anticoagulation was started in patients having SVT immediately after the diagnosis with warfarin with or without enoxaparin. Median length of anticoagulation with warfarin was 5.3 months. This is an ongoing study so results may vary in final set of data.

CA62

A different clinical profile of DOAC-treated patients: results from the real life cohort study

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Background: The use of DOAC is increasing for stroke prevention in patients with non-valvular atrial fibrillation (NVAF). The rate of stroke is described around 1.11%-1.7%. However, clinical trials patients are carefully selected and some risk factors could result in lower rates of therapeutic failure.
Aims: Determine the percentage of stroke in "real life" population treated with DOACs, and try to characterize the clinical profile of the patient.
Methods: We included pacientes with DOAC from June 2010 to November 2015. Risk factors for stroke were registred. We used CHA2DS2-VASc, HASBLED scores and renal function was calculated by Cockroft-Gault method.
Results: A total of 563 patients were recruited and 87.9 % (n = 495) had indication for NVAF. Mean age was 74.9 years (range: 40–93) and 53.5% were female. Among them, 158 (31.92%) were under dabigatran, 229 (46.26%) rivaroxaban, and 108 (21.82%) apixaban. Mean CHADSVASC score was 4.39, with HASBLED of 2.89. We found 144 patients with a glomerular filtration rate < 50 ml/min. Thirteen patients (2.6%) suffered a stroke. The mean CHA2DS2- VASC score was 5.69 with female predominance (11:2), mean age was 70.5 and all of them had hypertension. Notably, 11 patients were receiving DOAC as secondary prophylaxis. Patients under dabigatran had a mean follow up of 36.65 months, rivaroxaban 19.07 and apixaban 12.79 months. Stroke occurred in 2 patients with dabigatran 75 mg, 4 dabigatran 110 mg, 2 dabigatran 150 mg, and 5 rivaroxaban at correct doses. We confirmed good adherence.
Regarding the subsequent management, 4 patients under rivaroxaban changed to dabigatran and only 1 to apixaban; patients with dabigatran 75 mg changed to AVK, patients with dabigatran 110 mg changed to higher doses of 150 mg.
Conclusions: We described a low rate of stroke in patients treated with DOACs, but further studies are necessary in order to identify very high-risk patients with special emphasis in those with high CHA2DS2-VASC score, previous stroke and a correct anticoagulation.

CA63

Rivaroxaban therapy for patients with pulmonary embolism in real world: a prospective, single center, open-label observation study

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Background: The effectiveness and safety of rivaroxaban in pulmonary embolism (PE) have been demonstrated by many trials. But more experience is required to fulfill the gap between the designed trials and complicated clinical situations in Asia.
Aims: To see he safety and effectiveness of Rivaroxaban.
Methods: A prospective cohort study in hospitalised patients in FuWai Hospital, for acute, confirmed PE between May 2013-Aug 2015. All the PE patients were treated with rivaroxaban referring to standard dose recommended by ESC. The extension of anticoagulation treatment was decided in according to predisposing factors, bleeding risks and D-dimer value.
1 month, 3 months, and then per-6 months follow-up were performed until 2 years after the episode to record symptomatic recurrence PE, continuous pulmonary hypertension (PH), bleeding, and all-cause death. The recurrence was confirmed by imaging evidence. PH was defined by echocardiography.
Results: Seventy patients were enrolled,with 62 years old as the median of age and the eldest was 82 years old.
Four(5.7%) patients were already diagnosed with CTEPH suffering a recurrent APE.
During the follow-up, symptomatic recurrence PE was recorded in 3 patients (4.2%, 1 patient was in 6 month later, 2 patient both in 9 month later).
Only one (1.51%) patient was confirmed as CTEPH during the long term follow up.
Twenty-two (31.4%) patient occurred non-major bleeding, most are vary slight. Five (7.1%) patient died during the follow-up. Three patients died from malignant (two of them were final diagnosed with cancer during follow-up). One death attribute combined dilated cardiomyopathy, and the detailed cause of the other death was unknown.
Conclusions: Patients with pulmonary embolism in real world were more complicated than clinical trail volunteers. The patients' lifespan could be shorter owing to complications. But the safety and effectiveness of Rivaroxaban still be proved, with none major bleeding and low recurrence rate (4.2%). The incident rate of CTEPH was only 1.51%.

Disseminated Intravascular Coagulation

DIC01

Porcine model of methicillin-resistant staphylococcus aureus (MRSA) sepsis-induced disseminated intravascular coagulation (DIC)

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Background: Sepsis-induced DIC is associated with a high mortality. Currently, no therapy exists for sepsis and/or DIC other than supportive care. Although the worldwide incidence of Gram positive bacterial infections, especially MRSA, is increasing, there is a paucity of published Gram positive sepsis models. Pathologically and hemodynamically relevant large animal models are now recommended prior to human clinical trials.
Aims: To validate a published model (Soerensen et al 2013) and establish a hemodynamic porcine model of MRSA sepsis-induced DIC.
Methods: Five four-week old pigs (8 kg) were implanted with jugular and carotid catheters and a telemetry device in a femoral artery for continuous hemodynamic monitoring. Four days later the pigs were injected intravenously with MRSA (USA300, TCH 1516 strain) doses of 5 x10^8 to 1 × 10^9 CFU/kg. Fluid resuscitation was given for heart rate > 50% or mean arterial blood pressure < 30% from baseline. Point-of-care complete blood count, prothrombin time (PT), activated thromboplastin time (aPTT), D-dimer, and fibrinogen were done (Siemens CA600) at pre-, 12, 24, 36, 48 and 60 h post-MRSA injection. Pigs were euthanized then autopsies performed.
Results: Four pigs were analyzed through 60 h. One pig died early by 36 h. (Figure 1). Average platelet counts decreased by 38% from baseline. PT, aPTT, D-dimer, and fibrinogen levels all increased over time. Histologic examination revealed vascular thromboses, infarctions and hemorrhages in the kidneys, lungs, liver and/or heart of 4 pigs. Two pigs had cerebral edema.
Conclusions: Our porcine model of MRSA sepsis showed evidence of platelet consumption, activated coagulation, vascular thromboses and organ injuries. Further characterization of this model is ongoing with the aim of having a pathologically and hemodynamically relevant large animal model to study DIC and evaluate potential therapeutic targets.

Figure 1. Platelet Counts, Prothrombin Time, Activated Prothrombin Time, D-dimer, and Fibrinogen levels at pre-, 12, 24, 36, 48, and 60h post Methicillin-Resistant Staphylococcus Aureus Innoculation in Pigs.

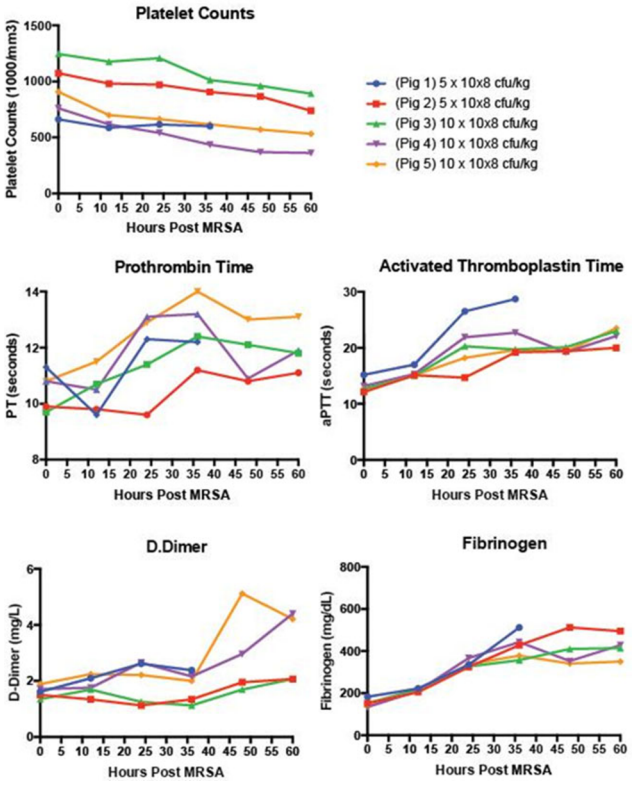

Figure 1 DIC Panel Pig MRSA.

DIC02

Histones released from extracellular nucleosomes upon dnase digestion are rapidly degraded by Factor VII activating protease (FSAP) to combat their cytotoxic effects

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Background: Animal studies have shown that injected histones are highly cytotoxic, and that anti-histone antibodies reduce lethality in sepsis models. High levels of circulating nucleosomes are frequently found in inflammatory disease, however it remains to be established whether histones are indeed present in a free, non-DNA bound form. The plasma protease Factor VII activating protease (FSAP) is autocatalytically activated upon contact with histones, whereafter histones are proteolyzed.
Aims: To determine the role of FSAP in the regulation of histone and/or nucleosome induced cytotoxicity.
Methods: Purified histones or nucleosomes were incubated with purified active or inactive FSAP, or healthy donor, FSAP deficient, or FSAP depleted serum, and added to HEK293 cells and LDH release detected. Moreover, we added benzonase DNase to digest DNA in nucleosomes and release histones, or added DNA to histones. FSAP activation was studied in ELISA. Histone proteolysis was studied on blot using anti-H3 antibody.
Results: Either active FSAP or endogenous FSAP in serum proteolyzed histone H3 and protected against the cytotoxicity of histones, whilst cytotoxicity remained using inactive FSAP, FSAP deficient, or depleted serum. We found that nucleosomes were hardly cytotoxic, unless DNase was added. Added DNA reduced the cytotoxicity of histones. FSAP inefficiently cleaved histone H3 when present in nucleosomes or with added DNA. Finally, we found that when nucleosomes were added to 6 healthy donor sera, DNase digestion led to FSAP activation and rapid degradation of histone H3 upon its release.
Conclusions: Our results suggest that FSAP safeguards against the highly increased cytotoxic effects of histones compared to nucleosomes. When free, non-DNA bound histones are inadvertently released, for instance when nucleosomes accumulate in disease and are targeted by plasma DNases, FSAP cleaves histones immediately upon their release to protect against their harmful effects.

DIC03

Laboratory tests as clues to understand the etiopathogenesis of hemostatic disturbances, acute kidney injury and mild intravascular hemolysis in *Bothrops* envenomation

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Background: Blood coagulation disorders are frequently recorded in patients following *Bothrops* snakebites, but clinical chemistry tests have been rarely reported. In fact, laboratory tests may be used to understand the pathophysiology of snake envenomation, as well as to improve the treatment of patients.
Aims: In this study we analyzed the records of usually requested laboratory tests from 114 patients bitten by *Bothrops* snakes.
Methods: Clinical chemistry, hematological and hemostatic analytes were evaluated in blood samples on admission (T_0) and/or at the first 24 h after antivenom therapy (T_1).
Results: Thrombocytopenia (45.6%), fibrinogen consumption (78.5%) and elevated D-dimer (91.6%) were observed in patients bitten by *Bothrops* snakes on admission. Bilirubin levels were increased in 36% of patients, with predominance of indirect bilirubin, and lactate dehydrogenase levels were raised in 66% of cases at T_0, suggesting the presence of mild intravascular hemolysis. Increased creatinine levels were detected in 14 of 106 patients (13.2%) at T_0 and T_1. Some subjects with normal creatinine levels at T_0 exhibited a marked rise during T_1. Acute kidney injury (AKI) was early and non-oliguric in most of these cases. AKI occurred more frequently in older patients, and even in mildly envenomed ones. AKI was associated with the severity of hemostatic disorders, intravascular hemolysis and systemic bleeding, and not with the severity of local manifestations.
Conclusions: *Bothrops* bites induce hemostatic disturbances, intravascular hemolysis and acute kidney injury (AKI). Thus, besides antivenom therapy, ancillary management (volume repletion) must be employed to prevent/ameliorate AKI.

DIC04

Tissue factor initiated thrombin generation in trauma patient plasma

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Background: Trauma patients tend to have an elevated procoagulant activity in plasma due, in part, to the presence of endogenous tissue factor (TF), factor (F)XIa and FIXa. Additionally, there is pronounced variability in endogenous procoagulants and coagulation inhibitors across individuals.
Aims: To evaluate thrombin (FIIa) generation (TG) in trauma patient plasma triggered with exogenous TF.
Methods: Trauma patient plasma samples from multiple time-points (222 samples from 64 patients), both with (n = 170) and without (n = 52) endogenous procoagulant activity, were evaluated for endogenous and 5pM TF-initiated thrombin generation (TG). Citrate plasma was prepared, frozen and stored at -80°C. No additional freeze/thaw cycles were involved prior to the activity assay.
Results: TG in plasma samples with endogenous activity and no exogenous TF varied in a wide range (see Table 1). An addition of exogenous TF had (almost) no effect on peak FIIa, but significantly reduced the lag phase. For these samples, TF levels were 0.27 + /-0.81pM (range 0 - >6.4pM), FXIa levels were 4.48 + /-10.01pM (range 0 - >64pM), and FIXa levels were 27.3 + /-115.7pM (range 0 - >1000pM).

Table 1

	Samples with endogenous activity				Samples without endogenous Activity (n = 51)*	
	0 pM Exogenous TF (n = 170)		5 pM Exogenous TF (n = 168)*		5 pM Exogenous TF	
	AVG (± SD)	Range	AVG (± SD)	Range	AVG (± SD)	Range
Lag Phase (s)	1574 (± 714)	159 - 3517	675 (± 308)	102 - 2000	946 (± 534)	254 - 2933
Peak IIa (nM)	218 (± 139)	22 - 614	243 (± 142)	26 - 622	161 (± 116)	27 - 483
Time to Peak (s)**	1750 (± 731)	358 - 3600	876 (± 327)	330 - 2295	1239 (± 654)	482 - 3446
AUC (nM*s)	83446 (± 38072)	5605 - 191897	116933 (± 37076)	5430 - 210577	89536 (± 33089)	5494 - 143304

*In 2 samples with endogenous activity and 1 sample without endogenous activity, no thrombin generation was observed in 3600 s (duration of the assay) upon addition of 5pM TF. These samples were excluded from analysis.
** If thrombin generation is observed but the peak value is not reached in 3600 s, then a value of 3600 s for time to peak is assigned to that sample.

Addition of TF to plasma samples with no endogenous activity also led to widely variable TG profiles. Of note, addition of TF to several plasma samples from coagulopathic patients did not lead to any measurable TG. Longitudinal samples from individual patients showed pronounced changes in FIIa parameters at different time points, with peak values varying >20-fold.
Conclusions:
1) TF-initiated TG varies across subjects and changes with time in individuals;
2) Addition of exogenous TF to trauma patient plasma with existing endogenous activity has a minimal (if any) effect on peak FIIa value, but usually shortens the lag phase to a varying extent;
3) Addition of TF to trauma patient plasma without endogenous activity leads to TG profiles with various durations of the lag phase and various peak values.

DIC05

Circulating histone as a marker of cardiovascular failure in patients with sepsis

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Background: Nuclear protein histone is released into the extracellular space during sepsis. Extracellular histone plays a role in the pathogenesis of sepsis in part through its toxic action against endothelial cells. However, there has been no reliable method for quantitative analysis of circulating histone levels other than semi-quantitative western blot analysis.
Aims: In this study we aimed to develop a sandwich ELISA to quantify serum/plasma histone H3 levels and to evaluate its clinical utility.
Methods: All experiments involving human subjects were approved by the Ethics Committee of Kagoshima University Hospital and Sapporo Medical University Hospital. Plasma samples were collected from 35 healthy volunteers and 110 patients who needed intensive care.
Results: The ELISA we developed detected histone H3 in serum and plasma of human, mouse, and rat origin with the working range of 10–250 μg/L. Plasma histone H3 levels in healthy volunteers were < 10 μg/

L whereas those in septic patients in the absence and presence of shock were 23.6 ± 32.0 μg/L and 93.7 ± 121.91 μg/L, respectively. Plasma histone levels correlated well with sequential organ failure assessment (SOFA) score, especially with its cardiovascular component.
Conclusions: Circulating histone levels are associated with cardiovascular failure in patients with sepsis. It is both possible that circulating histone exacerbates cardiovascular failure or that cardiovascular failure may induce histone release from damaged cells.

DIC06

Coagulopathy assessment in severe sepsis and septic shock patients. an observational pilot study

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Background: Hypercoagulability was described in sepsis leading to microcirculatory and organ failure despite abnormal standard coagulation tests(SCTs).
Aims: The aim of this study was to compare hemostasis in severe sepsis/septic shock patients with different disease severity evaluated by APACHE II score after ICU admission.
Methods: Adult patients with of severe sepsis/septic shock syndromes were included in the study group. Exclusion criteria:liver diseases, chronic kidney or hematologic diseases, pregnancy, anticoagulant/antiplatelet therapy, blood derivates or procoagulant treatments in the last 7 days. SCTs, individual coagulation factors plasmatic levels (PL) and rotation thromboelastometry (ROTEM® Germany) were performed in patients in the first 24–36 h after ICU admission. Following ROTEM parameters were calculated from the first derivative of the clot firmness curve:Maximum Velocity (MaxVel) and Time to Maximum Velocity of clot formation (t-MaxVel), Area under the curve (AUC) (Sorensen et al,2003). MCE(Maximum clot elasticity) was used to calculate δMCE, a parameter reflecting the platelet component of clot strength (Solomon C,2015).
Results: After Ethics Committee approval,51 patients were included in the study and were divided in 2 groups: 23 patients with APACHEII≥25 (HG) and 28 patients with APACHEII< 25 (LG). Non-significant differences were noted in SCTs, platelet number, PL and ROTEM parameters clotting time (CT), clot formation time (CFT), MaxVel and t-MaxVel between patients in HG and LG. Patients in HG had enhanced clot formation and strength- higher MCF, AUC and greater platelet contribution to clot stability- higher δMCE compared to LG.

Table

Test	LG Mean(± SD) or Median (Min, Max)	HG Mean(± SD) or Median (Min, Max)	P value (t test or Mann Whitney U test, * p statistically significant)
Fibrinogen (mg/dl)	295.85 (±128)	403.1 (±122.37)	P = 0.095
Platelets (per μL)	86500 (10000, 359000)	152500 (66000,409000)	P = 0.189
MCF (mm)	52.25 (±12.44)	65.91 (±7.41)	P = 0.007*
CT (sec)	82 (51, 192)	63.5 (53, 99)	P = 0.438
CFT (sec)	160 (76, 179)	93 (45, 188)	P = 0.056
MaxVel	13.5 (±5.52)	17.66 (±5.74)	P = 0.694
t-MaxVel	86.5 (53, 301)	72.5 (58, 172)	P = 0.84
AUC	5204.75 (±1220.16)	6547.33 (±745.22)	P = 0.007*
δMCE	103.93 (±50.42)	182.58 (±65)	P = 0.03*

Conclusions: Even if there are no significant differences in hemostasis activation, patients with increased disease severity demonstrate enhanced clot firmness with higher platelet contribution to clot strength as compared to patients with lower severity scores.For firm conclusions, the completion of this pilot study is required.

DIC07

Coagulation changes in porcine model of sepsis induced by methicillin-resistant staphylococcus aureus (MRSA) inoculation: correlation between ROTEM and routine laboratory tests

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Background: The incidence of MRSA pneumonia is increasing in patients worldwide and MRSA sepsis mortality is about 20–50%. Therefore, large animal models of MRSA induced sepsis are useful to investigate pathophysiological mechanisms and new therapeutic strategies.
Aims: The aim of our study was to evaluate the usefulness of ROTEM to detect hemostatic changes in the course of MRSA sepsis in porcine model and to compare with conventional coagulation tests.
Methods: Citrated blood was collected from 5 healthy 4-week old pigs (8 kg weight) at pre- and 60 h post MRSA intravenous inoculation. INTEM, EXTEM and FIBTEM were running on ROTEM, while PT, APTT, fibrinogen (FGN) and D-dimer were measured by Siemens CA600 coagulometer using commercial reagents. Data analysis was performed using paired t-test and Pearson's correlation (MS Excel). Data were presented as mean ± SD with significance at $P < .05$.
Results: Only 4 pigs were analyzed through 60 h; 1 pig died in 36 h. During the time-course of sepsis there was significant prolongation of PT and APTT accompanied with CT prolongation on EXTEM and INTEM with excellent PT vs CT EXTEM and APTT vs CT INTEM ($r = 0.94$, $P < .001$) correlations. FGN levels almost tripled and platelet (PLT) count dropped by 1/3 and accompanied with doubled increase of D-dimer. However, baseline MCF FIBTEM overestimated FGN due to incomplete porcine PLT blockade by cytochalasin or physiologic piglet thrombocytosis. Simultaneous FGN increase and PLT count drop during septic course resulted only to modest increase of MCF in EXTEM and FIBTEM. Of interest, ML increased on EXTEM and INTEM, reflecting fibrinolysis activation or clot retraction.

Table 1

Coagulation tests	PT, sec	APTT, sec	FGN, mg/dL	D-dimer, mg/L	PLT, x10^3/μL
Baseline	10.3 ± 0.6	12.7 ± 0.5	144 ± 10	1.61 ± 0.24	996 ± 209
60 h later	12.0 ± 0.8	22.2 ± 1.5	422 ± 59	3.21 ± 1.32	631 ± 231
ROTEM tests	CT EXTEM, sec	CT INTEM, sec	MCF FIBTEM, mm	MCF EXTEM, mm	ML EXTEM,%
Baseline	38 ± 2	95 ± 9	56 ± 4	78 ± 1	4 ± 1
60 h later	48 ± 4	141 ± 19	67 ± 4	84 ± 1	10 ± 0

Conclusions: Changes in routine coagulation tests during 60 h of MRSA-induced sepsis in pigs fulfilled all human criteria for DIC except FGN level. ROTEM analysis can be useful to monitor hemostatic changes in this model including fibrinolysis activation; however FIBTEM is not suitable for accurate FGN estimation.

Fibrinolysis

FIB01

Hyaluronic acid decreases the mechanical stability, but increases the lytic resistance of fibrin

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Background: Hyaluronic acid (HA) is a large, non-sulfated glucosaminoglycan abundantly present at sites where fibrin is also formed (during wound healing, in arterial restenotic lesions and eroded atherosclerotic plaques). The co-localization of HA and fibrin raises the possibility for their interaction with consequent modifications in the mechanical and lytic properties of the clots.
Aims: To characterize the structure of composite fibrin-HA clots, their viscoelastic properties and susceptibility to fibrinolytic proteases.
Methods: Scanning electron microscopy (SEM), pressure-driven permeation and small-angle X-ray scattering (SAXS) for fibrin structure. Oscillation rheometer for viscoelastic properties. Kinetic turbidimetric and chromogenic assays for dissolution of fibrin and plasminogen activation by tissue-type plasminogen activator (tPA).
Results: Fibrin formed in the presence of native (1,500 kDa) HA and its 500 kDa fragments had thicker fibers and larger pores according to the SEM and clot permeation data, whereas the 25 kDa HA fragments had only minor effects (Table 1). SAXS evidenced a mild dearrangement of protofibrils. These structural alterations suggest that HA modifies the pattern of fibrin polymerization so that lateral association of protofibrils dominates over formation of branching points. The rheometer data indicated that softer fibrin structures were formed with 1,500 kDa and 500 kDa HA and these clots presented with lower dynamic viscosity values and gel/fluid transition at lower critical stress values (τ_0) (Table 1). tPA-catalyzed plasminogen activation was markedly inhibited by HA, both in free solution and on the surface of fibrin clots (Table 2). HA of 1,500 kDa and 500 kDa size prolonged clot lysis with both plasmin and tPA and this inhibition was kringle-mediated, because 6-aminohexanoate abolished it and it was not observed with des-(kringle1–4)-plasmin.
Conclusions: Our data suggest a role for HA in the stabilization of fibrin at sites of tissue injury and inflammation.

Table 2 (Abstract FIB02)

	Additive at 0.5 mg/ml			
	None	1,500 kDa HA	500 kDa HA	25 kDa HA
In solution	0.192 (0.017)	0.071 (0.012)*	0.050 (0.005)*	0.050 (0.011)*
On the surface of composite fibrin	0.776 (0.154)	0.499 (0.109)*	0.579 (0.106)*	0.360 (0.045)*

Plasminogen activation in the presence of HA. Plasminogen activation by tPA was followed by continuous measurement of the cleavage of Spectrozyme-PL, a plasmin substrate in solution or on the surface of fibrin clots. Mean and standard deviation of plasmin generation rates (n = 5–10) are shown in nM/min units, asterisk indicates $P < 0.05$ according to Kolmogorov-Smirnov test in comparison to the basal activation rate in the respective assay in the absence of HA.

FIB02

Thrombolysis - proteolysis - isopeptidolysis

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Background: For the long time clinical and scientific worldwide publications showed, that thrombolysis is the proteolytic degradation of fibrin-polymers. The role of isopeptide bonds was not clear. The situation changed radically after the discovery of medicinal leech enzyme Destabilase, which hydrolyse ε-(γ-Glu)-Lys isopeptide bonds in stabilized fibrin. Now we prepared soluble recombinant enzyme cultivated in *E.coli* cells.
Aims: Our aim was to investigate fibrinolytic properties of Destabilase *in vitro* and *in vivo* compared to Streptokinase and Urokinase.
Methods: *In vitro* study was conducted on plates of stabilized fibrin. Saline and the test substances were dispensed to the plates; 24 h later the diameter of dissolved zones was measured. *In vivo* study was conducted with 46 male Sprague Dawley rats (weight 450–550 g). Arterial (carotid artery) and venous (jugular vein) thrombi formation in anesthetized rats was stimulated with 10% $FeCl_3$. The test substances were injected into the tail vein 24 h after the thrombi formation: Saline (0,4 ml), Streptokinase (3,5 mg/kg), Destabilase (0.8 mg/kg). After next 24 h thrombi were removed, dried and weighed. During the *in vivo* study Destabilase activity was detected in blood (L-γ-Glu-pNA).
Results: The diameter of zones dissolved by Destabilase was 60% larger than Streptokinase or Urokinase. The compound of Destabilase and Urokinase increased zone diameter by 140%. *In vivo* Destabilase administration decreased weight of thrombi vs. saline- and Streptokinase-treated rats in both venous and arterial models. Destabilase decreased arterial thrombus stabilization degree threefold compared to saline and Streptokinase.

Table 1 (Abstract FIB01)

		Additive at 0.2 mg/ml			
		none	1,500 kDa HA	500 kDa HA	25 kDa HA
Fiber diameter from SEM (nm), median (bottom-top quartile)		86.3 (70.5–104.3)	97.2* (78.0–119.4)	98.6* (80.6–120.5)	80.4* (65.8–98.1)
Fluid permeability coefficient from clot permeation assay (Ks, 10^{-9} cm^2), mean (standard error)		0.71 (0.05)	1.14* (0.09)	1.07* (0.11	0.86* (0.03)
Viscoelastic parameters	G' storage modulus (Pa)	34.05 (8.31)	22.21* (5.5)	18.23* (4.33)	24.19 (6.40)
From oscillation rheometer	G'' loss modulus (Pa)	3.10 (0.51)	2.28* (0.52)	1.98* (0.39)	2.48 (0.50)
Mean (standard deviation)	Loss tangent (G''/G')	0.092 (0.009)	0.104* (0.009)	0.110* (0.011)	0.104 (0.011)
	τ_0 (in relative units compared to pure fibrin)	1.00 (0.13)	0.65* (0.12)	0.79* (0.15)	0.81 (0.15)

Structural characteristics of fibrin/HA clots. The critical shear stress (τ_0), an indicator of the gel/fluid transition in the fibrin structure was determined by extrapolation of the fall in viscosity to 0 when clots were exposed to increasing stress in oscillation rheometer. Asterisks indicate $P < 0.05$ according to Kolmogorov-Smirnov test in comparison to pure fibrin, n = 4–12.

Conclusions: Hydrolysis of isopeptide bonds by Destabilase results in spontaneous transition of the stabilized fibrin-polymer from solid to soluble state. Destabilase can perform thrombolysis independently or provide additional opportunities to proteolytic enzymes by reducing the degree of fibrin stabilization and the revelation of previously inaccessible areas.

FIB03

polyphosphate co-localises with FXII on platelet-bound fibrin and markedly augments αFXIIa-mediated plasminogen activation

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Background: Platelet polyphosphate (polyP) binds factor XII (FXII) and acts as a surface for autoactivation and activation by kallikrein. Activated FXII (αFXIIa) is a known weak plasminogen activator.
Aims: To determine the effects of polyP on αFXIIa-mediated plasminogen activation.
Methods: αFXIIa-mediated plasminogen activation and fibrinolysis ± $polyP_{70}$ (average polymer chain length) were assessed by chromogenic assay and clot lysis respectively. FXII binding analyses were performed by western blotting of flow-through material from columns containing polyP coated sepabeads. Confocal microscopy and flow cytometry were utilized to detect DL488-labelled FXII or polyP (DAPI) binding to washed platelets. Plasma clots were formed ± washed platelets ± DAPI and DL488-FXII.
Results: αFXIIa-mediated fibrin clot lysis (105 ± 6.5 min vs. 238 ± 14.4 min; $P < 0.0001$) and plasminogen activation ($P < 0.0001$) were significantly augmented by $PolyP_{70}$. αFXIIa did not directly influence plasmin activity. $PolyP_{70}$ bound aFXIIa but not βFXIIa that lacks the surface binding domain. In line with this, βFXIIa is a poor plasminogen activator. αFXIIa-mediated clot lysis was enhanced to a similar degree (2.6-fold) with Glu- and Lys-plasminogen; suggesting αFXIIa does not facilitate transition of the closed (Glu) to open (Lys) form. αFXIIa-plasminogen activation was enhanced by soluble fibrin but not by FXII activators RNA and collagen. The plasminogen activator function of αFXIIa is down-regulated by C1 inhibitor and histidine-rich glycoprotein, in contrast, PAI-1 and PAI-2 did not modulate αFXIIa-mediated clot lysis or plasminogen activation ± $polyP_{70}$. DL488-FXII bound clearly to platelets and the adjacent fibrin network. Similarly, platelet-derived polyP associated with the platelet surface and bound surrounding fibrin where it co-localised with FXII.
Conclusions: In the presence of platelet polyP and fibrin aFXIIa is a potent plasminogen activator which may be relevant *in vivo*.

FIB04

Recombinant human ADAMTS13: first-in-human study evaluating pharmacokinetics, safety and tolerability in hTTP patients

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Background: Hereditary thrombotic thrombocytopenic purpura (hTTP, or Upshaw Schulman Syndrome), is a rare, life-threatening microvascular disease characterized by extensive formation of platelet-rich microthrombi due to ADAMTS13 deficiency. ADAMTS 13 is currently replaced using FFP or specific intermediate purity Factor VIII concentrates.
Aims: To evaluate the pharmacokinetics (PK), safety and tolerability of novel recombinant human ADAMTS13 (rADAMTS13) in severe hTTP.
Methods: A phase 1, multicenter, open-label, dose-escalation clinical study was conducted at 9 sites (US=1, Europe=6, and Japan=2). A total of 15 subjects, 12–65 years, with severe hereditary ADAMTS13 deficiency (< 6% activity by FRETS-VWF73 [1] assay) received a single infusions of rADAMTS13 at doses of 5 (n = 3), 20 (n = 3) and 40 (n = 9) U/kg BW. Presented are data from the initial 10 subjects, aged 17–41 years: 5 males, 5 females; 8 receiving regular prophylaxis (7 FFP, 1 BPL-8Y), 2 treated episodically. Pre-existing anti-ADAMTS13 antibodies or immunosuppressive medication administration were exclusions. Study data were reviewed by an external DMC after completion of dosing cohorts 1 and 2 and prior to enrollment of the final 5 subjects into dosing cohort 3.
Results: PK parameters were estimated from the first 4 subjects treated with 40 U/kg. The median IR was 0.022 U/mL per kg/U (range 0.013 to 0.0.26); median $T_{1/2}$ was 57.9 h (range: 44.1–70.6 h); and median AUC was 41.8 (range 31.2–79.1) h U/mL. Activity measurements by FRETS and chromogenic assay methods were highly comparable. C_{max} values obtained from the 3 dosing cohorts demonstrated a linear dose response that suggests dose proportionality. There were no related serious or non-serious AEs, and no binding or inhibitory anti-ADAMTS13 antibodies detected related to rADAMTS 13 infusion.
Conclusions: rADAMTS13 infusion was safe and well tolerated in hTTP patients with PK parameters comparable to those estimated from FFP studies.

FIB05

The covalent or non-covalent modification of streptokinase with polyethylene glycol improves its properties

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Background: The instability to proteolysis, rapid clearance and high fibrinogenolytic effect restrict the use of streptokinase(SK) in thrombolytic therapy.
Aims: Improving the properties of SK by non-covalent and covalent modification with polyethylene glycol (PEG).
Methods: (i) Solution of SK with albumin was encapsulated in PEG 20 or 40 kDa (SK/PEG_{cap}) by double emulsification method and sonification (22 kHz).
(ii) SK was conjugated with activated PEG 2 or 5 kDa ($SK\text{-}PEG_{con}$). Release of SK from SK/PEG_{cap} and its stability in human plasma were traced using Christensen method. Lysis of plasma clots and depletion of plasma fibrinogen by SK/PEG_{cap}, $SK\text{-}PEG_{con}$ and free SK were measured *in vitro*.
Results: (i) Four SK/PEG_{cap} preparations of various compositions (d 7 - 10 μm) were obtained by varying the concentration and MW of PEG. They contained 90 - 95% of the active SK and fully released it in 45, 60, 75 and 90 min. All SK/PEG_{cap} (500 IU/ml) induced 100% clots lysis in 4 h, like SK, but they caused lesser fibrinogen depletion ($\tau_{1/2}$ 35, 38, 40 and 41 min) than SK ($\tau_{1/2}$ 9 min). (ii) By varying the reaction time of SK with activated PEG, optimal $SK\text{-}PEG2_{con}$ and $SK\text{-}PEG5_{con}$ preparations retained 80% of SK activity were obtained. $SK\text{-}PEG2_{con}$ and $SK\text{-}PEG5_{con}$ (200 IU/ml) were more stable in plasma ($t_{1/2}$ 45 and 52 min), than SK ($t_{1/2}$ 22 min). The k_{cat}/K_m of plasminogen activation by SK, $SK\text{-}PEG2_{con}$ and $SK\text{-}PEG5_{con}$ were compared. The $SK\text{-}PEG2_{con}$ and $SK\text{-}PEG5_{con}$ had high thrombolytic activity (85 and 65%) and caused 3.5 - 4 fold smaller fibrinogenolysis, than equal dose of SK.

Conclusions: Both the encapsulation in HMW PEG and conjugation with LMW PEG improves the SK properties. SK inside PEG-microcapsules is protected from proteolysis and gradually freeing into the plasma causes slower fibrinogenolysis. In the SK-PEG conjugates, the PEG molecules, linked to ε-amine groups of lysine's of protein, interfere with the proteolysis of SK and degradation of fibrinogen by SK.

FIB06

Inhibitory effects of intact heavy chain of plasminogen and its fragment K1-4.5 on the generation of plasmin *in vitro*

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Background: Angiostatin K1-4.5 comprising kringles 1–4 and 85% of kringle 5 of heavy chain of plasminogen (Pg) inhibits angiogenesis and tumor metastasis. We earlier showed that K1-4.5 inhibits Pg activation, and this is one of the mechanisms of its antiangiogenic action because plasmin plays a central role in extracellular proteolysis, cell migration and tube formation.
Aims: Comparison of inhibitory effect of K1-4.5 and intact heavy chain of Pg (K1-5r) containing intact kringles 1–5 and connecting peptide (r) on Pg activation.
Methods: The K1-5r and K1-4.5 were obtained from human Pg and their influence on Glu-Pg activation by uPA in the absence and by tPA in the presence of soluble fibrin was measured with conjugated method.
Results: The K1-4.5 and K1-5r had no effect on amidolytic activities of plasmin, tPA and uPA, but they inhibited the Pg activation with uPA and tPA in dose-dependent manner. Inhibition constants of Pg activation (K_i) with K1-4.5 and K1-5r were equal to 0.59 ± 0.02 and 0.12 ± 0.01 μM for uPA and 0.40 ± 0.03 and 0.050 ± 0.003 μM for tPA, respectively. Influence of K1-4.5 and K1-5r on kinetic parameters (k_{cat} and K_m) of Pg activation with uPA and tPA was studied. The K1-4.5 and K1-5r had no effect on k_{cat} and have increased K_m of Pg activation with uPA, whereas they have reduced the k_{cat} and have increased the K_m of fibrin-stimulated Pg activation with tPA. Thus, K1-4.5 and K1-5r inhibit Pg activation with uPA by competitive type and with tPA by mixed type.
Conclusions: The proposed mechanism of inhibition: the K1-4.5 and K1-5r due to the presence of kringle domains displace Pg from the uPA·Pg complex, whereas in the case of tPA, they displace Pg from the tPA·Pg complex and from fibrin surface. Reducing the concentration of active tPA·Pg or tPA·Pg·fibrin complexes leads to a decrease in the plasmin generation rate. Thanks to kringle 5 integrity, the K1-5r is a more strong inhibitor of plasmin generation and, probably, of angiogenesis than angiostatin K1-4.5.

FIB07

The time course of plasma procarboxypeptidase U (proCPU, proCPB2 or TAFI) in traumatic brain injury and aneurysmal subarachnoid hemorrhage is not associated with the presence of acute coagulopathy

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Background: Traumatic brain injury (TBI) and subarachnoid hemorrhage (SAH) are the main indications for admission to the neurocritical care unit. Although being heterogeneous and complex, both pathologies are often associated with the development of an early systemic coagulopathy. To further support the role of the fibrinolytic system in this poorly understood condition, we investigated the role of procarboxypeptidase U (proCPU, proCPB2 or TAFI). ProCPU is a zymogen that after release of its activation peptide, plays a role in clot stabilization.
Aims: To investigate the time course of proCPU in patients with TBI and SAH. Correlation with routine hemostatic parameters and association with acute coagulopathy was assessed.
Methods: Blood samples were collected from 20 TBI and 25 SAH patients over a time-course of 12 days. Plasma proCPU concentrations were measured using a previously described HPLC-assisted assay. Additionally, fibrinogen, platelet count, aPTT and INR were assessed. Acute coagulopathy was defined as an INR >1.5 and/or aPTT >40s and/or platelet count < 100,000/μL within 24 h. A mixed effects modeling approach was used to analyze longitudinal data. Holm-Bonferroni correction was applied for multiple comparisons.
Results: We found low proCPU levels (mean 634 U/L, range 237–1049 U/L at 36 h; normal range 602–1363 U/L) in the first 72 h after onset of TBI and SAH, followed by a gradual but pronounced rise of proCPU up to day 12 (mean 1351 U/L, range 632–2070 U/L at 288 h). ProCPU was positively correlated with platelets [log(proCPU) vs log (PLT count); $R = 0.199$, $P = 0.01$] and negatively with INR [log (proCPU) vs 1/INR; $R = 0.183$, $P = 0.02$]. However, acute coagulopathy was not associated with a difference in plasma proCPU ($P = 0.16$).
Conclusions: Taken together, proCPU is decreased in the acute phase of TBI and SAH, which is most likely the result of consumption in the first hours after the event. ProCPU levels were correlated with platelet count and INR, but there was no association with the presence of acute coagulopathy.

FIB08

Procarboxypeptidase U (proCPU, TAFI, proCPB2) in cerebrospinal fluid (CSF) in the hyperacute phase of ischemic stroke

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Background: Carboxypeptidase U (CPU, CPB2 or TAFIa) is a potent attenuator of fibrinolysis. Elevated plasma levels of its precursor proCPU have been linked to acute ischemic stroke (AIS).

The decrease in plasma proCPU concentration in the first 72h after stroke onset correlates with stroke severity, evolution and outcome. So far, nothing is known about proCPU in CSF of stroke patients but recently proCPU has been detected in CSF of control individuals.
Aims: In this study we evaluated the presence of proCPU in CSF from patients in the hyperacute phase of ischemic stroke and its relationship towards stroke parameters and blood-brain barrier (BBB) dysfunction.
Methods: A sensitive in-house HPLC assisted assay was used to analyze proCPU levels in CSF of non-thrombolysed patients in the hyperacute phase (< 24h after onset) of AIS (n=72). Samples were collected in the Middelheim's Interdisciplinary Stroke Study. 15 individuals with no apparent abnormalities in biochemical and microbiological tests, served as controls. ProCPU levels were expressed as mean ± SD. A Mann-Whitney U-test was used to evaluate differences between groups. Relations between proCPU levels and ordinal data were assessed by Spearman's rho (ρ). Pearson r was used for continuous variables.
Results: No significant differences were found in proCPU levels in CSF of patients with AIS (4.3 ± 1.9 U/L) compared to control individuals (3.1 ± 1.6 U/L). ProCPU levels in CSF of AIS patients correlate (p=0.04) with stroke outcome expressed by the modified Rankin Scale at month 3 and the mortality rate. Furthermore, biomarkers of BBB dysfunction (e.g. the albumin index) correlated ($P< 0.05$) with proCPU levels in CSF of stroke patients.
Conclusions: ProCPU is present in CSF from stroke patients as well as in CSF from control individuals. However, proCPU concentrations in both populations were not significantly different. The correlation of proCPU concentrations in CSF of stroke patients with outcome parameters is possibly secondary to the extent of BBB dysfunction.

FIB09

Local hemostasis changes and endothelial damage during percutaneous transcatheter isolation of the pulmonary veins in patients with atrial fibrillation

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Background: Left atrial ablation (ABL) with pulmonary vein isolation (PVI) is an established treatment of atrial fibrillation (AF). Possible complications of ABL include cerebral embolism.
Aims: To find out whether ABL is associated with local, intracardiac alterations of hemostasis/fibrinolysis and to analyze different ABL techniques in this respect.
Methods: Study population included 44 consecutive paroxysmal/persistent AF patients undergoing ABL. PVI was performed using the cryoballoon (CB) in 24 and the phased radiofrequency technology with the circular, multipolar pulmonary vein ablation catheter (PVAC) in 20 patients. All medications influencing coagulation were discontinued at least 5 days before ABL. Blood samples were taken from the femoral vein and the left atrium (LA) before and after ABL. During ABL iv heparin was administered (100IU/kg). FXIII activity, plasminogen activity, plasminogen-antiplasmin (PAP) complex, D-dimer, fibrin monomer (FM), plasminogen activation inhibitor-1 (PAI-1) activity and antigen level, soluble VCAM-1 and soluble E selectin (sEsel) were measured from all blood samples. Informed consent was obtained from all patients.
Results: Before ABL, significantly elevated PAP complex, D-dimer and FM levels were found in the LA samples as compared to those of the femoral vein. When LA samples of pre- and post-ABL were compared, significantly lower FXIII, plasminogen, PAI-1 activities and FM levels as well as markedly elevated D-dimer levels were found post-ABL, suggesting increased clotting and fibrinolysis. When the two ABL technologies were compared, significantly lower plasminogen levels and higher PAP complex levels were found in case of PVAC. The presence of endothelial damage was demonstrated by the significantly elevated sVCAM-1 and sEsel levels in PVAC post-ABL LA samples, however, no such alteration was found in case of CB procedure.
Conclusions: PVAC ABL seems to be associated with increased fibrinolysis and endothelial damage as compared to the CB procedure.
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FIB10

Thrombin activatable fibrinolytic inhibitor (TAFI) increases in the plasma of cancer patients with prothrombotic behavior

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Background: Venous thromboembolic disease risks are elevated in tumoral patients. Despite this knowledge, anticoagulation regimes are limited due to the tumoral pro-hemorrhagic risk. Identification of those highly prothrombotic patients become really important. This study relates on a biobank collection of tumoral patients from recent diagnosis, before their initiation of antitumoral treatment. This collection possesses highly controlled conditions both in terms of blood extraction, sample isolation and preservation, and quantitation assays.
Aims: Identify plasma biomarkers from highly prothrombotic risk tumoral patients.
Methods: A collection of plasma samples from cancer patients has been recruited with their signed consent and both, the study and biobank collection, approved by the Ramón y Cajal Hospital Ethics Committee. Plasma from tumoral patients or healthy individuals was isolated from citrated blood. Obtained samples were frozen, and patients clinical data kept in a more than 400 different individual parameters database. Patients were also analyzed for their Khorana′s scale risk and their clot-lytic impairment assay, using a pure fibrin clot covered by exogenous tPA concentration in the presence of control or tumoral plasma. Based on these levels, we subsequently measured TAFI levels by ELISA assay (Asserachrom®) kindly provided by STAGO S.A.D.
Results: 63 patients identified as impaired fibrinolytic capacity, belonged to a highly prothrombotic tumor types such as pancreas, lung, non-Hodgkin lymphomas, colorectal or breast cancers. Most of these patients felt down in a Khorana VTE risk degree above 0, being most of the tumors 2 or above. While healthy control plasmas showed clot-lysis assay above 70 %, tumoral plasmas could be classified in levels between 60–70%, 50–60 % and below 50% for the same kinetic time. Interestingly, TAFI levels inversely correlated with fibrinolytic impairment.
Conclusions: This work presents TAFI as a potential biomarker to associate with the prothrombotic risk in cancer patients.

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FIB11

Combinatorial therapy with C1 esterase inhibitor and tranexamic acid to treat hemorrhagic shock

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Background: About one third of all severely injured patients has trauma induced coagulopathy and hemorrhagic shock, which causes a high mortality. Most effective treatments, such as 1:1:1 transfusion protocol (a combination of packed red blood cells, fresh frozen plasma and platelets) are only available at the points of a qualified medical care. In treating hemorrhagic shock conditions, it is extremely important to implement therapeutic measures as early as possible. In many circumstances the qualified medical care is too late for the effective treatment. Therefore, emergency medicine units need an effective therapeutic agent to treat hemorrhagic shock and trauma induced coagulopathy.
Aims: To show the effect of combined therapy tranexamic acid with C1 inhibitor esterase in the treatment of hemorrhagic shock.
Methods: Experimental animal models of acute blood loss and hemorrhagic shock; Thromboelastography; Inhibition of inflammatory cytokines release ex vivo/in vitro; Carrageenan induced edema.
Results: We showed the survival improvement of the experimental animals in the model of acute blood loss and hemorrhagic shock with the combined therapy using C1 esterase inhibitor and tranexamic acid. This combination was significantly more effective than standard crystalloid infusion therapy or each of the active components alone. We showed evidences that the possible mechanism of action of the used combination may be associated with the suppression of coagulopathy and hyperfibrinolysis (showed using the model of secondary fibrinolysis in vitro). Moreover, the combined therapy in the rodent model of hemorrhagic shock effectively prevents intestinal epithelium damage.
Conclusions: The combined therapy of C1 esterase inhibitor and tranexamic acid is significantly more effective in the model of acute blood loss and hemorrhagic shock than standard crystalloid infusion therapy.

FIB12

Effect of elapsed time between plasma separation and processing on fibrinolytic activity

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Background: Due to tissue activator plasminogen instability, fibrinolytic activity tests can be affected by preanalytic variables.
Aims: It was to evaluate the effect of elapsed time between plasma separation and processing, as well as the effect of freezing on euglobulin lysis time (ELT).
Methods: Plasmas (n=36) were tested by ELT (M.Kowalski). Platelet poor plasma (PPP) was immediately obtained by double centrifugation of blood samples (9:1 in acid citrate, pH 5). Six aliquots were obtain: 1 for basal testing, 3 were immediately frozen (t0), 2 were frozen after 1h (t1) or 2h (t2) in ice bath water. Aliquots t0 were processed after 1, 5 or 10 days at -20°C ($t0_1$, $t0_5$ y $t0_{10}$); t1 and t2 were processed after 1 day at -20°C. Statistics: Normality was verified by D'Agostino-Pearson's test; t-Student-paired samples or Wilcoxon tests were applied to compare parametric or nonparametric variables.
Results: *Freezing and thawing:* Not significant differences ($p_{Wilcoxon}$= 0.48) were found between basal testing and $t0_1$ results. Aliquot $t0_1$ was considered as reference for other comparisons.
Delayed processing: t1 and t2 results were compared vs. $t0_1$; significant differences were obtained ($t0_1$-t1: $p_{t\text{-}Student}$< 0.0001; mean=22.3m; IC95%=12.8–31.9 and $t0_1$-t2: $p_{Wilcoxon}$< 0.0001; media=32.5m). Moreover, TLE prolongation was directly proportional to the interval length between PPP obtention and freezing (t1–t2: $p_{t\text{-}Student}$= 0.0004; mean=14.8m; IC95%=7.4–22.2).
Time at -20°C: $t0_5$ and $t0_{10}$ results were compared with those of $t0_1$ aliquot; significant differences were obtained ($t0_1$–$t0_5$: $p_{t\text{-}Student}$=0.18; mean=8.5m; IC95%=4.4–21.5 and $t0_1$–$t0_{10}$: $p_{t\text{-}Student}$=0.29; mean= 6.0m; IC95%= 5.7–17.8).
Conclusions: Freezing and thawing, when PPP was obtained immediately after blood withdrawal and consecutively frozen, did not affect ELT. Not significant changes were observed in samples preserved up to 10 days at -20°C. However, the delay in either sample processing or freezing, modified ELT.

FIB13

Longitudinal analysis of coagulant and fibrinolytic markers of trauma patients that received tranexamic acid

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Background: Recent trials (CRASH-2) advise the early administration of tranexamic acid (TXA) to bleeding trauma patients.
Aims: Evaluate the coagulant and fibrinolytic potential of trauma patients that received TXA following a trauma using static and dynamic assays.
Methods: Trauma patients admitted to the emergency department of the Mayo Clinic had blood drawn and citrated plasma prepared at various time points over the initial 72 h. Patients that received TXA were identified and plasma samples were subsequently analyzed for α-thrombin•antithrombin (αTAT), fibrin monomer (F-monomer), plasma•antiplasmin complex (PAP), D-dimer, and neutrophil elastase•α1-proteinase inhibitor complex (NE-α1PI). Dynamic measurements of coagulant and fibrinolytic potential were measured using a thrombin generation assay and a modified clot lysis assay (CLA).
Results: Two of six patients who received TXA were subsequently diagnosed with a DVT. Across all patients and all time points: D-dimer levels ranged from 5 to 128-fold above upper limit of normal (ULN) and NE-α1PI ranged from 30–190 ng/mL. 0-h measurements of F-monomer ranged from 4–125-fold above ULN and 1–10-fold above ULN for PAP. For 5 of 6 patients, 0-h measurements of αTAT ranged from 69–347-fold above ULN. CLAs conducted in the presence of tissue factor (TF) -/+ tissue plasminogen activator (tPA) stimulus reveal the fibrinolytic potential of samples and the residual presence of TXA following therapeutic administration. Patients diagnosed with a DVT had high sustained levels of D-dimer (> 28-fold above ULN) and average NE-α1PI levels > 110 ng/mL across all time points, a secondary surge in αTAT detected at 24 h, and prolonged resistance to tPA initiated lysis through 24 h.
Conclusions: Monitoring both coagulant and fibrinolytic markers and clot lysis potential of patient samples following TXA administration may serve as a predictor for subsequent DVT risk.

Factor VIII, Factor IX & Rare Coagulation Disorder

FVIII01

INSIGHT case-control study: high FVIII concentrate dose and surgery are associated with increased inhibitor risk in nonsevere hemophilia a patients

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Background: Inhibitor development is a major complication of treatment with factor VIII concentrates in nonsevere hemophilia. Studies including severe hemophilia A (HA) patients have shown that intensity of treatment is an important treatment-related risk factor, however evidence on risk factors in nonsevere HA patients is scarce. It is important to identify clinical situations that elicit inhibitor development, as this enables the design of preventive strategies.
Aims: We investigated the association between intensity of treatment and inhibitor development in nonsevere HA patients.
Methods: This nested case-control study includes 75 inhibitor patients (cases) and 223 controls, selected from 2709 nonsevere HA patients (FVIII:c 2–40%) of the INSIGHT study. Cases and controls were matched for date of birth and cumulative number of exposure days (ED) to FVIII concentrates. A conditional logistic regression model was used to calculate both unadjusted and adjusted hazard ratios; the latter adjusted for a-priori specified confounders.
Results: This analysis includes 298 patients, with a median age at first exposure of 23 years (inter quartile range (IQR) 5–44) and a median FVIII baseline level of 8 IU/dL (IQR 4–14). The 75 cases developed an inhibitor after a median of 25 ED (IQR 12 - 40).
Peak treatment of 5 EDs or 10 EDs did not increase inhibitor risk (aHR 1.0, 95% CI 0.4–2.5 and HR 1.8, 95% CI0.6–5.5 respectively). History of a surgical intervention increased the inhibitor risk 4-fold (aHR 4.295% CI 1.7–10.3) and a high mean dose (>45 IU/kg/ED) of FVIII concentrate strongly increased inhibitor risk (aHR 7.5, 95% CI 1.6–35.6) compared to a mean dose of $\leq$ 25 IU/kg/ED.
Conclusions: In this nested case-control study in nonsevere HA patients, a high dose of FVIII concentrate and surgery were both associated with an increased risk of inhibitor development.

FVIII03

FVIII-LRP1 interaction affinity is biased by FVIII concentration

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Background: Coagulation factor (F) VIII circulates in plasma at low concentrations and is bound to von Willebrand factor (VWF) with high affinity (K_D < 1 nM). Only a small portion of FVIII (2–5%) circulates as free protein. LRP1 (low density lipoprotein receptor-related protein 1) has been identified as an important clearance receptor. As long as bound to VWF, FVIII is protected from direct interaction with LRP1. Several reports on the binding affinity of FVIII to LRP1 with K_D values of ~80 nM are available. In light of the low plasma concentration of FVIII (~0.3 nM), this affinity is relatively weak. However, binding data were generated using purified proteins and analyzed at supra-physiological FVIII concentrations up to >100 nM.
Aims: We determined FVIII-LRP1 interaction using a broad FVIII concentration range using state-of-the art surface plasmon resonance (SPR) equipment.
Methods: Interaction kinetics of FVIII to LRP1 was measured using Biacore T200 technology, which allowed measurement even at very low FVIII concentrations. Subsequent to immobilization of recombinant LRP1 cluster II or plasma derived LRP1 on sensor chip, recombinant FVIII was injected. The FVIII concentration ranged from 0.6 to 100 nM.
Results: When measuring FVIII-LRP1 interaction in the high FVIII concentration range (10 to 100 nM), K_D values in the double digit nM range (to 10^{-8} M) were determined. This is in accordance to previously reported data, where measurements were performed at comparable concentrations. Experiments at lower concentrations (below 1.6 nM) revealed much higher binding affinities: K_D values were in the subnanomolar range, 10^{-10} M, which is close to the physiologic FVIII plasma concentration.
Conclusions: In summary, the data show that FVIII-LRP1 binding affinity depends on the FVIII concentration, suggesting that under physiological conditions, low local concentrations of FVIII allows LRP1 binding with high affinity. This is in accordance with the rapid clearance of VWF-unbound FVIII, present at low concentrations.

FVIII04

Variation in baseline Factor VIII concentration in mild and moderate hemophilia a patients carrying the same *F8* mutation

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Background: Mild and moderate hemophilia A (MHA) patients with higher baseline factor VIII concentrations (FVIII:C) report less traumatic joint bleeds. The underlying gene mutation is the most

important determinant for the residual level of FVIII:C and consequently bleeding phenotype. Furthermore, von Willebrand Factor (vWF) levels, blood group and age also influence bleeding variability. Despite the current availability of mutational analyses techniques, the pathophysiological understanding of the causative genetic event leading to reduced FVIII:C levels and bleeding complications is incomplete.

Aims: To assess the effect of mutation, age, vWF and blood group on FVIII:C in MHA patients.

Methods: Patients were selected from the INSIGHT and the RISE study, including data of 3534 MHA patients from Europe, Canada and Australia. To reduce selection bias, we selected patients ≥ 10 years from centers in which more than 70% of the patients was genotyped for routine care. Missense mutations present in ≥10 patients were analyzed. The FVIII:C levels were measured by one-stage clotting assay in the local center. Mean FVIII:C was used in patients with multiple available FVIII:C measurements . Multiple linear regression was used to analyze the association between FVIII:C, *F8* genotype, vWF, age, and blood group.

Results: Twelve different missense mutations were present in ≥10 patients (n=346) from 8 hemophilia treatment centers (HTCs). Median FVIII:C was 15 IU/dL (IQR 9-23). Mutation, age, vWF, blood group and HTC together explained 59% (adjusted R^2) of the variance in mean FVIII:C levels in this population. Four of the 12 missense mutations had no effect on FVIII:C (Arg2169His, Leu644Val, Pro149Arg and Val502Gly).

Conclusions: We observed four *F8* missense mutations that did not significantly associate with FVIII:C levels in this population of MHA patients. This awareness is important in diagnostic patient management, since patients with the same mutation might have variable FVIII:C levels.

FVIII05

A retrospective study of the current treatment practice of haemophilia A and B in France

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Background: Haemophilia treatment practice varies significantly between individuals, countries and regions, and bleeding and treatment data are often incomplete, hampering the planning of future healthcare resource allocations.

Aims: To provide information on current FVIII/FIX treatment practice in Montpellier and Nantes, France.

Methods: This study is part of a larger, non-interventional, 12-months retrospective study exploring factor treatment for haemophilia A and B in several European countries. For France, anonymized data were retrieved from patient charts. Male patients on factor treatment 24 months prior to inclusion in this study, with basal FVIII/FIX levels ≤5 IU/dL and without inhibitors were included. Data were summarized descriptively.

Results: In total, 102 patients with haemophilia A and 29 with haemophilia B were included, 69% and 41% had severe disease, respectively. Prophylaxis and on-demand treatment were equally common for haemophilia A although prophylaxis was more prevalent among children. On-demand treatment was most common (76%) for haemophilia B. Most haemophilia A patients on prophylaxis were treated thrice (47%) or twice (43%) a week. Most haemophilia B patients were treated twice a week (71%). The mean (SD) prescribed prophylactic treatment was 91.9 (27.2) IU/kg/week (n=46) for haemophilia A and 97.7 (32.1) IU/kg/week (n=6) for haemophilia B. For those with available bleeding data (A; n=81, B; n=19), all patients on prophylaxis, except one each with haemophilia A and B, experienced ≥1 bleed. The median annual bleeding rate was 4.0, both for patients with severe haemophilia A and B on prophylaxis and for joint bleeds it was 2.5 for haemophilia A and 1.0 for haemophilia B.

Conclusions: These data provide an updated view on the current treatment practice for patients with haemophilia A and B at two treatment centres in France, illustrating that patients on prophylaxis still bleed, emphasizing a need for further advancing the standard of care.

FVIII06

Non-neutralizing antibodies against Factor VIII and the risk of inhibitor development in untreated and minimally treated patients with severe hemophilia A (SIPPET study)

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Background: development of anti-FVIII neutralizing antibodies is the most important complication in the management of patients with hemophilia A. Non-neutralizing antibodies (NNAs) may be detected in patients with hemophilia A without an inhibitor (INH) as well as in unaffected individuals.

Aims: to evaluate the role of NNAs as risk factor for INH development.

Methods: This was a cohort study performed in the frame of SIPPET, in which previously untreated or minimally treated (< 5 exposures to blood components) children affected with severe hemophilia A were followed until inhibitor development, 50 EDs or end-of-study date. two-hundred forty-five plasma samples collected before any exposure to FVIII concentrates were analyzed for detection of NNAs and INH. NNAs were detected by anti-human IgG HRP polyclonal antibody, after being captured on a full length recombinant FVIII with a home-made ELISA assay. The cut-off was set at >1.035 ug/ml of specific anti FVIII IgG. INH was monitored using Bethesda assay (cut off ≥ 0.4BU).

Results: At screening no INH was detected whereas NNAs were found in 44/245, with a strong age gradient. Of the 44 cases with NNAs, 18 (16 high-titre) subsequently developed an INH, for a cumulative incidence of 45.8% (CI95 29.7–61.9%) whereas in those without NNAs, 57 (34 high-titre) out of 201 developed an INH, for a cumulative incidence of 33.4% (CI95 26.1–40.7%). In univariate Cox regression patients with NNAs had a 69% higher incidence of INH development than patients without NNAs (HR 1.69, CI95 0.99–2.87). For high-titre INH the rate was more than 2-fold increased (HR 2.44, CI95 1.35–4.42). The associations did not materially change after adjustment for putative confounders (age, country, class of treatment, previously exposure to blood components): adjusted HR 1.97 (CI95 1.10–3.53) for any INH, and HR 3.03 (CI 95 1.54–5.96) for high-titre INH.

Conclusions: NNAs before any exposure to FVIII concentrate are frequently present and associated with an increased incidence of INH, including high-titre INH.

FVIII07

Immunological profile of previously untreated patients with haemophilia a: results from the HEMFIL study

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Background: The development of inhibitors is the most serious complication in patients with hemophilia. Previous cross-sectional studies evaluated the effect of factor VIII replacement on the immunological profile of hemophilia A (HA) patients, but no study investigated this in previously untreated patients (PUP).

Aims: This study aimed to compare the immunological profiles of PUP with HA and healthy, non-hemophiliac boys.

Methods: A panel of chemokines, cytokines and microparticles (MPs) were evaluated in 32 PUP with HA and 47 controls by flow cytometry. The control group was composed of healthy, non-hemophiliac boys, with normal clinical examination; weight, BMI and renal function within reference range; normotensive; and no morbid past worthy of note (Table 1). All parents signed a written informed consent.

Results: PUP had higher plasma levels of CXCL8/IL-8 (95% confidence interval [CI], 1.98 - 2.64; $p < 0.01$), IL-6 (95% CI, 0.99 - 1.93; $p < 0.01$), TNF (95% CI, -0,72 - 0,44; $P = 0.02$), IL-4 (95% CI, -0,72 - 0,22; $P = 0.01$), IL-10 (95% CI, -0,04 - 0,83; $p < 0.01$), IL-17 (95% CI, -0,84 - 0,52; $p < 0.01$) and lower levels of CXCL9/MIG (95% CI, -0,46 - 1,53; $P = 0.03$) when compared with controls (Figure 1). We also observed higher levels of MPs (MPs/μL) derived from endothelium (95% CI, 0,76 - 1,02; $p < 0.01$), erythrocytes (95% CI, 1,00 - 1,24; $p < 0.01$), platelets (95% CI, 1,26 - 1,84; $p < 0.01$), leukocytes (95% CI, 0,70 - 1,18; $P = 0.01$), neutrophils (95% CI, 0,62 - 0,84; $p < 0.01$), and T lymphocytes (95% CI, 0,65 - 0,87; $p < 0.01$) in PUP when compared with the control group.

Conclusions: PUP with HA and controls have distinct immunological profiles expressed by different levels of cytokines, chemokines and MPs. We hypothesize that this immunological stimulation might result from impaired hemostasis in PUP with HA due to the deficiency of factor VIII. This study was supported by Fapemig and CNPq.

Table 1 Characteristics of the study population.

	PUP (n=32)	Controls (n=47)
Age, median (IQR)	8 months (2.8–11.3)	12.2 months (7.7–16.7)
White	22 (68.8)	35 (74.5)
Black	5 (15.6)	9 (19.1)
Mixed	4 (12.5)	–
Indian native	1 (3.1)	–
Unknown	–	3 (6.4)
Motive of diagnosis, *n* (%)		
Bleeding	26 (81.3)	not applicable
Family history	6 (18.7)	not applicable

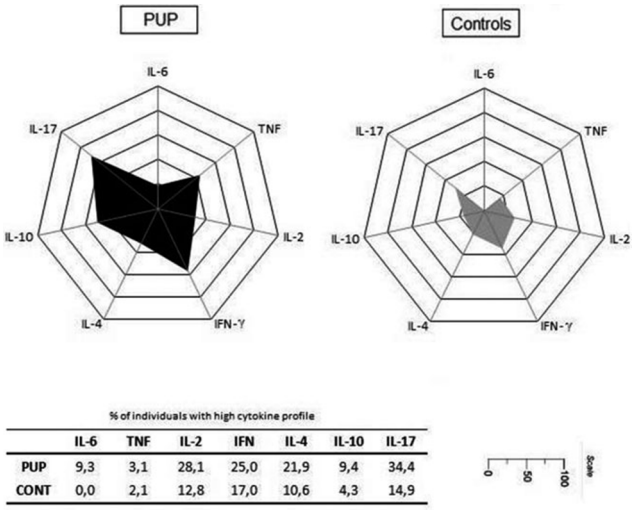

Figure 1. Radar graphical representation of cytokine patterns in patients and controls
This chart summarizes the percentage of high cytokines balance in previously untreated patients (PUP, black area), and controls (gray area). Each axis displays the proportion of each cytokine balance category.

FVIII08

Thrombin generation assay (TGA) for monitoring the haemostatic response in patients with hemophilia and inhibitors on immune tolerance induction (ITI): preliminary in vitro and in vivo results from the predicTGA study

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Background: Whether TGA may predict haemostatic efficacy of different FVIII concentrates in patients with hemophilia (FVIII ≤2 IU/dl) and inhibitors remains unclear.

Aims: This prospective multicenter study was set up to evaluate TGA using full-length rFVIII (FLR), B-domain deleted rFVIII (BDDR) and plasma-derived FVIII/VWF (PD). The study was approved by local ethic committees and informed consent obtained from all patients.

Methods: Centralized laboratory assessment: TGA (CTA Thrombinoscope. Maastricht, NL), FVIII by one-stage assay and inhibitor by Nijmegen-Bethesda assay. Patients underwent baseline assessment and prospective follow-up (12–33 month) with FVIII and TGA testing before and after FVIII administrations.

Results: 41 patients (age 1–72 year) from 18 centers were recruited. Statistically significant differences in TGA parameters were shown between-concentrates in baseline spiking experiments; e.g., median

peak-thrombin was highest after spiking with PD (80, 2–288 nM) and lowest with FLR (44, 0–206 nM), $P< 0.001$. These results paralleled the inhibitor titer when measured against each of the 3 concentrates. Preliminary data are available from 12 patients who completed at least 12-month follow-up or terminated the observation period. ITI started in 11 high responders (with PD in 6, FLR in 4, BDD in 1) and prophylaxis with PD in 1 low responder. Post-infusion FVIII and peak thrombin values were correlated with inhibitor titer (r:-0.73, p=0.007 and r:-0.76, p=0.004, respectively). A correlation was also found between post-infusion peak thrombin and FVIII values (r: 0.61, p=0.034).

Conclusions: In baseline in vitro tests the PD concentrate resulted as the least reactive against inhibitors and the most efficient in generating thrombin. In inhibitor patients receiving FVIII treatment TGA showed to be sensitive in distinguishing different responses. Analyses are underway to assess correlation of in vitro and in vivo data, hemostatic response and ITI outcome.

FVIII09

Importance of standardized joint-ultrasound for individualization of prophylaxis in hemophiliacs: easy-to-learn-ultrasonography (HEAD-US) of joints and correlation with function in haemophilic arthropathy - results of a clinical trial

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Background: In Haemophilic Arthropathy the extent of synovitis is detected as a sign of the activity, osteochondral defects are quantifiable as a sign of progression by ultrasound. In 2013 an easy-to-use standardized ultrasound protocol (HEAD-US) for examination of early joint changes in Hemophilic Arthropathy was published by C. Martinoli. This is the first clinical trial to correlate clinics, functional and structural changes in Haemophilic Arthropathy.

Aims: Easy-to-use and highly standardized sonographic quantification of the Haemophilic Arthropathy by haemophilia-treaters could be the basis for better individualized therapy in young Haemophiliacs.

Methods: In 2012 - 2015 we have included more than 200 young german patients with hemophilia A or B or vWD from different German haemophilia treatment centers in the HaemarthroSonoPilot trial (DRKS00004483, informed consent, ethical approved by the LÄK Baden-Württemberg). Standardized Ultrasound of the elbow, knee and ankle joints was performed and rated with the HEAD-US scale in each patient. Simultaneously an orthopedic clinical examination with clinical scoring and 3D motion analysis of the lower limbs for detecting early function defects (rolling vs. gliding in motion) were performed with an ultrasonic topometer.

Results: The investigations in the presented pilot study showed correlation of the sonographic diagnostics with the measurement of a clinical orthopedic examination in haemophilic arthropathy depending on age. Through the joint sonography changes were even partially already seen before that stood out in the clinical investigation.

Conclusions: It may be useful if haemophilia treaters in future apply an easy to learn standardized ultrasonography (HEAD-US) to individualize the therapy under close control and evaluation of joint changes.

FVIII10

Evaluation of the efficiency of immunotolerance in hemophilia a: preliminary results of the Brazilian immunotolerance study

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Background: Immune tolerance induction (ITI) is the treatment of choice to eradicate inhibitors in patients with hemophilia A (HA) who develop persistent inhibitors against infused factor VIII (FVIII). In Brazil, ITI has been recently implemented.

Aims: To present preliminary data on the efficiency of ITI.

Methods: Eligible patients had severe/moderate HA with persistent inhibitors (≥2 measurements of inhibitor ≥5 Bethesda units [BU]/mL) and needed bypassing agents. There was no exclusion based on age at start of ITI, time since inhibitor development or historical peak. Most patients initiated ITI when inhibitor titre was ≤40 BU/mL. The protocol consisted of 50 international units (IU) per kilogram (kg) of FVIII concentrate (either plasma derived [pd] or recombinant [r]) 3 times a week. Upon lack of response the dose was increased to 100 IU per kg daily. All patients/guardians signed a consent form. The study was supported by Fundo Nacional de Saúde.

Results:

Table 1 Number of patients included in ITI per year.

Year of Inclusion	Number of patients included
2011	7
2012	47
2013	58
2014	70
2015	58
Total	240

Source: Hemovida Web Coagulopatias, Ministry of Health, Brazil 2016

Between October 2011–December 2015, we included 240 patients (Table 1). All were male, median age of 7 years (range 1–49) at start of ITI. This represented 56.1% of eligible patients (n =428). A total of 188 (78.3%) and 52 (21.7%) patients received pdFVIII and rFVIII concentrate, respectively. A total of 81/240 (33.8%) patients completed ITI. Of these, 65 (80.2%) patients responded to ITI, of whom 39 (59.6%) and 26 (35.8%) patients had complete and partial response, respectively. Sixteen (19.8%) failed ITI after 33 months of treatment. A total of 17/240 (7.1%) abandoned ITI. Analysis on the efficiency of the ITI according to the type of factor concentrate, dose and potential confounding are underway.

Conclusions: We conclude that low-dose ITI eradicated inhibitors in 80% of Brazilian patients with HA, who were not selected as "good-risk" based on parameters influencing the success of ITI. Since this report relates to 30% of enrolled patients who concluded ITI, a complete analysis on the efficiency of the ITI will require a longer follow-up.

FVIII11

A novel Factor VIII-nanobody fusion protein displaying prolonged half-life & haemostatic efficacy in vivo

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Background: Clearance of factor VIII (FVIII) is strongly dependent von Willebrand factor (VWF), a phenomenon that limits half-life

prolongation of long-acting FVIII variants. Bypassing this limitation by using long-acting VWF variants is compromised because FVIII that is co-infused with such long-acting VWF variants is redistributed to endogenous VWF, due to the rapid association-dissociation kinetics that characterize the VWF-FVIII interaction (Yee at al. (2014) Blood 124:445).

Aims: We aimed developing a FVIII protein that resists dissociation from long-acting VWF variants.

Methods: Two copies of a non-inhibitory nanobody recognizing human and murine VWF-D'D3 domain displaying slow dissociation kinetics (10^{-6} s^{-1}) were engineered into the FVIII protein, replacing the FVIII B-domain. Cell lines expressing this variant (FVIII-KB013bv) were established, and purified protein was analyzed for in vitro activity. Half-life and efficacy in a tail-bleeding model were determined using *F8*-deficient mice.

Results: FVIII-KB013bv was produced as a single-chain protein of 190 kDa, and thrombin-activation released the nanobody fragment, generating the classic heavy and light chain derivatives. FVIII-KB013bv was fully active in 1-stage clotting and 2-stage chromogenic assays (Act/Ag ratio being 1.0 and 1.1, respectively). In vivo analysis revealed a 1.9-fold increase in half-life compared to control B-domainless FVIII (p=0.0032). Blood loss determined in a tail-clip assay 24 h after a single infusion (500 U/kg) was significantly reduced in mice receiving FVIII-KB013bv compared to those receiving B-domainless FVIII (13±3 microL vs 194±146 microL; n = 5–6; P = 0.0043), confirming the long-acting capacity of FVIII-KB013bv.

Conclusions: This prototype FVIII-nanobody fusion protein represents a novel approach to improve hemophilia A treatment. Combination of this protein with long-acting VWF variants is anticipated to prolong its half-life well beyond the limit of the current long-acting FVIII variants.

FVIII12

ELISA combined chromogenic assay (ECA) for determination of binding of PEGylated rFVIII to VWF

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Background: Von Willebrand factor (VWF) forms a high-affinity complex with coagulation factor VIII (FVIII), which stabilizes and protects FVIII from early degradation and cellular uptake. Baxalta has developed an extended half-life recombinant FVIII (rFVIII) modified with polyethylenglycol (PEG). Binding of PEG-rFVIII to VWF is an important functionality to be characterized and controlled.

Aims: Here, we describe the development and qualification of a simple method to measure the binding of PEG-rFVIII to VWF.

Methods: The VWF-FVIII binding (VWF:FVIIIB) assay is based on a combination of ELISA and a chromogenic assay for FVIII (VWF: FVIIIB ECA). A constant amount of recombinant VWF (rVWF) is mixed with a dilution series of PEG-rFVIII and incubated for a defined period. The formed complex of PEG-rFVIII and VWF is then transferred to a microtiter plate, where an anti-human VWF antibody is immobilized. After incubation, unbound PEG-rFVIII is removed by washing and PEG-rFVIII bound to VWF quantified using reagents of a FVIII chromogenic assay. Samples VWF-FVIII binding is calculated relative to a rFVIII reference standard assumed to have 100% VWF: FVIIIB.

Results: PEG-rFVIII concentration dependently bound to VWF in a working range of 0.0125–0.4 IU/mL FVIII activity. VWF:FVIIIB of PEG-rFVIII was >85% of that of the rFVIII reference standard, confirming intact VWF binding properties of PEG-rFVIII. Intermediate precision was determined from repeat measurements of three lots of PEG-rFVIII in nine independent test units, with a mean coefficient of variation of 15%, indicating good assay precision. The coefficient of determination of the single dilution point fitting for the rFVIII reference was ≥0.99, indicating acceptable linearity. Various control experiments confirmed the assay's specificity.

Conclusions: In conclusion, the VWF:FVIIIB ECA allows simple and rapid determination of PEG-rFVIII binding to VWF with good precision, and is thus an important analytical tool to characterize a new extended half-life rFVIII product.

FVIII13

The patient reported outcome burdens and experiences (PROBE) study - phase 1 results show PROBE study methodology feasible

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Background: Healthcare payers desire to better understand health outcomes. Patient-reported outcomes often differ from clinical endpoints. Patients' knowledge, perspectives and experience can contribute to defining and measuring key outcomes. The Patient Reported Outcomes, Burdens, and Experiences (PROBE) study develops a new global tool to respond to this emerging need.

Aims: The PROBE study aims to develop and validate a standardized survey to gather experiential data reported by patients and collected by patient organizations to support evidence-based recommendations to entities responsible for making healthcare reimbursement decisions.

Methods: In Phase 1 a questionnaire was developed, refined and tested for content, relevance, clarity and completeness. Methodology and feasibility were assessed. The PROBE questionnaire incorporates EQ-5D-5L with additional domains identified by patients: treatment and bleeding history, pain, independence, schooling, employment, relationships and activities of daily living.

Results: Phase 1 fieldwork is complete. 704 responses were received from 17 participating countries (117% of study objective), demonstrating feasibility. These included 77 mild, 77 moderate and 276 severe hemophilia patients, as well as 274 controls with no bleeding disorder. Among other findings, 72% of severe patients reported at least one "target joint" when asked a generic question, "Do you currently have any 'target joints'?" 77% reported reduction in range of motion in at least one joint. However, only 44% answered yes to having a "target joint" according to the more traditional definition, 2 or more spontaneous bleeds into a joint in the past 12 months.

Conclusions: Limitation in range of motion is a common and important patient reported outcome and closely correlates to patients' concept of a "target joint". Future phases of PROBE will provide valuable global perspectives through patient-reported health outcomes and experiences.

FVIII14

The relationship between thrombin generation and spontaneous bleeding for hemophilia a subjects in A-LONG and extension study

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Background: The one-stage clotting assay (OS) is widely used for determining coagulation activity and dosing requirements during FVIII replacement therapy. Specific for FVIII, this assay does not reflect a patient's global hemostatic balance. A thrombin generation assay (TGA) may provide more relevant insight into a patient's coagulation potential.
Aims: To explore the relationship between thrombin generation and spontaneous bleeding in severe hemophilia A subjects enrolled in the A-LONG and extension study (ASPIRE).
Methods: Seventy-four subjects on a tailored prophylaxis regimen received an initial dose of 50 IU/kg rFVIIIFc for PK and TGA assessment. Logistic regression was used to explore the association between spontaneous bleeding events (≥ 1 vs. 0 over a 1-year observation period) and peak thrombin production or FVIII activity.
Results: An association between spontaneous bleeding and the maximum peak thrombin level was found (p=0.0045, odds ratio 0.686): every 10-unit increase in maximum peak thrombin was associated with a 31.4% reduction in the odds of bleeding. Similar relationship was also found between spontaneous bleeding and the differential peak thrombin generation (pre-dose vs. max post-dose peak thrombin) (p=0.013, odds ratio 0.711). When the maximum post-dose FVIII activity was included in the model as a single factor or a covariate, no association was found with spontaneous bleeding.
Conclusions: Our results showed that higher post-dose peak thrombin generation is associated with lower risk of bleeding in patients with severe hemophilia A but not peak FVIII activity by OS. The change in peak thrombin generation between pre- and post-dose (i.e. additional thrombin generated in response to a rFVIIIFc dose) also correlated with bleeding tendency. These results indicate that a standardized TGA may be a useful tool for predicting bleeding risk in subjects with otherwise equivalent dosing optimization based on FVIII activity.

FVIII15

Internal consistency and item-total correlation of patient-reported outcome (PRO) instruments and hemophilia joint health score v2.1 (HJHS) in US adult people with hemophilia (PWH): results from the pain, functional impairment, and quality of life (P-FiQ) study

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Background: Assessment of pain and functional impairment in PWH is inconsistent in the clinical setting. Standardized and disease-specific PROs and HJHS have been used in studies but not validated in adult PWH.
Aims: To assess internal consistency (IC) and item-total correlation (ITC) of PROs and HJHS in PWH.
Methods: Adult male PWH with joint pain/bleeding completed 5 PROs (EQ-5D-5L with visual analog scale, Brief Pain Inventory v2 Short Form [BPI], International Physical Activity Questionnaire [IPAQ], SF-36v2, and Hemophilia Activities List [HAL]) and underwent a musculoskeletal exam (HJHS). IC was evaluated by Cronbach's alpha (>0.70 considered satisfactory) and ITC by Pearson's product-moment correlation (>0.20 considered satisfactory).
Results: P-FiQ enrolled 381 PWH; median age was 34 years. On EQ-5D-5L, Health Index IC was 0.81; ITC was higher for Mobility (0.70), Usual Activities (0.71), and Pain-Discomfort (0.73) than for Self-Care (0.53) and Anxiety-Depression (0.39). On BPI, IC was 0.92 for Pain Severity and 0.96 for Pain Interference; ITC was high for all component scores (0.79 to 0.90). On IPAQ, IC of Total Physical Activity was 0.51; ITC was higher for Walking (0.50) and Moderate Intensity Activities (0.67) than Vigorous Activities (0.12). On SF-36v2, IC ranged from 0.79 (Vitality) to 0.96 (Role Physical, Role Emotional, Physical Health Summary, and Mental Health Summary); within Vitality, lowest ITC was for Feel Full of Life (0.56). On HAL, all domains had high IC (0.89 to 0.98) except for Use of Transportation (0.58); within this domain, ITC ranged from 0.34 (Using Public Transportation) to 0.49 (Riding a Bicycle). On HJHS, Total Score IC was 0.97; ITC of Total scores ranged from 0.34 (Global Gait) to 0.70 (Atrophy, Right; Flexion Loss, Right).
Conclusions: IC was generally high, with lowest values for IPAQ Total Physical Activity and HAL Use of Transportation. Instrument scores related to pain/physical function appear to contribute more to consistency than those of self-care and anxiety/depression.

FVIII15A

Known-group validity of patient-reported outcome (PRO) instruments and hemophilia joint health score v2.1 (HJHS) in US adult people with hemophilia (PWH): results from the pain, functional impairment, and quality of life (P-FiQ) study

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Background: Pain and functional impairment (FI) due to joint disease are major problems affecting adult PWH. Standardized and disease-specific PROs have been used in studies of PWH to assess pain and FI but not validated.
Aims: To assess known-group validity of PROs and HJHS in adult PWH.
Methods: During routine visits, males with moderate-severe hemophilia and a history of joint pain/bleeding completed a pain history and 5 PROs: EQ-5D-5L, Brief Pain Inventory v2 Short Form (BPI), International Physical Activity Questionnaire (IPAQ), SF-36v2, and Hemophilia Activities List (HAL); HJHS was performed by a physical therapist. Scores were compared to known groups of interest based on self-reported characteristics (**see Table**) and assessed by Wilcoxon Rank-Sum Test; significant ($P < .05$) results are reported.

Table

	Known Groups of Interest
Anxiety/depression	Yes; no
Pain	Acute; chronic; both
Lifetime EoRF	Never; 25%-49%; 50%-74%; 75%-99%; always
FI	Unrestricted/full; limited/no activity
Arthritis/bone/joint problems	Yes; no
Current treatment regimen	On-demand; prophylaxis
Hemophilia severity	Severe; mild/moderate

Results: P-FiQ enrolled 381 PWH; median age was 34 years. On EQ-5D-5L, Anxiety/Depression discriminated known anxiety/depression groups, and Pain/Discomfort discriminated known pain and lifetime

extent of routine factor infusion (EoRF) groups. On BPI, all Pain domains discriminated known pain groups and Pain Interference discriminated known FI groups. On IPAQ, Walking discriminated known EoRF groups, and Moderate and Vigorous Activities discriminated known arthritis groups; Total Activity discriminated known FI, arthritis, and current treatment regimen groups. On SF-36v2, Physical Functioning, Bodily Pain, and Mental Health discriminated known FI, pain, and anxiety/depression groups, respectively; Physical Functioning and Bodily Pain also discriminated EoRF. On HAL, all scores discriminated known FI and EoRF groups. On HJHS, Global, Ankle, and Gait scores discriminated known arthritis, EoRF, current treatment regimen, and hemophilia severity groups; Knee and Elbow scores discriminated known arthritis, EoRF, and hemophilia severity groups.
Conclusions: All 5 PROs and HJHS demonstrate known-group validity. The use of specific instruments should be tailored to study design or clinical need for specific outcome assessment.

FVIII16

HLA genotyping and Factor VIII intron 22 inversion in children with severe haemophilia a: relation to Factor VIII inhibitor development

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Background: The development of inhibitors against factor VIII (FVIII Inh) in Haemophilia A (HA) patients is the most serious complication of treatment, rendering replacement therapy ineffective. HLA alleles, cytokine polymorphisms and FVIII gene null mutations and inversions are included among genetic predisposing factors.
Aims: To investigate the impact of HLA-A, B, DRB1 and DQB1 alleles on the risk for Inh development in a larger than previously published group of Greek children with severe HA (n:83), exclusively treated with recombinant products, and also to correlate Inh formation with intron 22 inversion, as an index genetic factor.
Methods: Thirty-nine children had developed inhibitors (Group I), while forty-four had not (Group II). Detection of intron-22 inversion was performed by Long Range PCR and HLA genotyping by PCR-SSOP (One Lambda) and PCR-SSP (Invitrogen, Genovision) methods. The data were statistically analyzed by Fischer's exact test reporting the corresponding odds ratios (OR) with their 95% confidence intervals (CI).
Results: The intron-22 inversion frequencies were significantly greater in Group I as compared to Group II (63.9% vs. 34.4%, p=0.028, OR=3.4 95% CI=1.2–9.2). Compared to Group II, in Group I increased frequencies of DRB1*01 (p=0.04, OR=4.1 95% CI=1.1–16.4), DRB1*01:01 (p=0.05, OR=46, 95% CI=1.0–23.6) and DQB1*05:01 (p=0.02, OR=4.7, 95% CI=1.2–18.6) were estimated. On the other hand HLA-DRB1*11:04 (p=0.03, OR=0.3, 95%CI=0.1–0.9) and HLA-DQB1*03 (p=0.03, OR=0.4, 95% CI=0.1–0.9) presented significantly lower frequencies. No correlation was found between HLA A, B alleles and Inh formation.
Conclusions: In conclusion, there is a significant evidence that HLA-DRB1*01, DRB1*01:01 and DQB1*05:01 are predisposing factors for FVIII inhibitor development in Greek children with haemophilia A. On the contrary, DRB1*11:04 and DQB1*03 confer resistance to Inh formation, findings with clinical significance for the treatment of the patients.

FVIII18

Clinical decision making is effective in reducing bleeding rates without the need to monitor FVIII trough levels tn patients with severe hemophilia a receiving prophylaxis with rVIII-SingleChain

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Background: A Phase III study investigated the efficacy of rVIII-SingleChain, a novel rFVIII product, in pediatric patients < 12 years of age with severe hemophilia A.
Aims: To investigate the effect of dose adjustments based on clinical decision making on bleeding rates.
Methods: Patients were assigned to a prophylaxis or on-demand therapy by the investigator. Patients on prophylaxis could be prescribed 15 to 50 IU/kg rVIII-SingleChain every second day or 2 to 3 times per week, or at other doses or dosing frequencies at the investigator's discretion. Dose and frequency could be adjusted during the study if necessary. Monitoring of FVIII levels was neither mandated nor routinely performed during the study.
Results: 84 patients were enrolled into the study, 81 were assigned to prophylaxis. Of those, 43 (53%) were assigned to a 2x weekly and 24 (30%) to a 3x per week regimen. The median ABR (annualized bleeding rate) across all prophylaxis regimens was 3.69 (Q1, Q3: 0.00, 7.20), the median AsBR (annualized spontaneous BR) was 0.00 (Q1, Q3: 0.00, 2.20). 49 patients on prophylaxis did not receive a dose adjustment while on study; these patients had an ABR of 2.73. Within this population, 5 children had ≥ 2 spontaneous bleeds within a 14-day period with no dose adjustment and had a median observed ABR of 6.94, compared to an ABR of 2.58 in the 44 prophylaxis patients with no dose adjustment who did not have ≥ 2 spontaneous bleeds within a 14-day period. 31 prophylaxis patients had at least 1 dose adjustment, the median ABR prior to dose adjustment was 7.83, the median ABR following the dose adjustment was 2.48.
Conclusions: Individualized dosing can result in low ABRs/AsBRs when dosing decisions are based on clinical bleeding phenotypes. The low ABRs for subjects who did not meet the clinical criteria for a dose adjustment or in whom the dose was adjusted when clinically indicated, is highlights the need for active monitoring of prophylaxis success in children with severe hemophilia A.

FVIII20

Introduction of a standardised heat inactivated Nijmegen - Bethesda Assay (NBA) for the detection and quantification of FVIII inhibitors

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Background: Development of FVIII antibodies is a complication of Haemophilia A or an auto immune response (Acquired Haemophilia A)[1]. BCSH guidelines recommend inhibitor testing at regular intervals using a Nijmegen-Bethesda Assay (NBA)[2] As external quality assurance surveys show high inter-laboratory variation, a standard NBA is recommended.[3,4] This includes buffering normal pooled plasma (BNPP), using FVIII deficient plasma as sample diluent and removing baseline FVIII by heat inactivation before analysis. [3,4]
Aims: Implement a standard NBA to meet best practice guidelines.[1,4]
Methods: The study group samples were from patients with congenital and acquired FVIII deficiency (n=37). Analysis by the NBA and the current method (18/37 positive and 19/37 negative for inhibitors) was performed. NBA included heat inactivation of plasma at 58°C for

1.5 h. Inhibitor positive samples were prediluted with FVIII deficient plasma. BNPP containing 1.0 IU/ml FVIII was prepared (0.1M Imidazole, pH 7.4). Samples were mixed with BNPP (1:1) and incubated for 2 h at 37°C. Post incubation, FVIII activity was measured and inhibitor level determined, taking account of dilution factors[2,3,4] Results for both assays were compared to assess NBA suitability for implementation.

Results: 19/19 tested negative, 17/18 tested positive with both assays. 1/18 (congenital severe FVIII deficiency) measured 0.5BU by our current assay and negative by NBA. 9/18 inhibitor positive samples showed linear kinetics by both methods and correlation (R^2 0.998). The remaining 9/18 with non-linear kinetics, were also comparable for each dilution with good correlation (R^2 0.999).

Conclusions: The NBA showed acceptable correlation with our current Bethesda method for patients with congenital and acquired Haemophilia A. It is suitable for implementation to standardise inhibitor detection in compliance with BCSH and other best practice guidelines. Results close to the detection limit (0.5BU) require evaluation and correlation with clinical findings.

FVIII21

Isolation of antibodies that differentiate between wild-type FIX and the padua variant

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Background: FIX Padua is a naturally occurring hyper-functional variant of wild-type FIX with a single amino acid exchange (FIX R338L). The usefulness of FIX Padua for hemophilia B gene therapy was shown in preclinical models and is currently being explored in Baxalta's BAX 335 clinical phase 1/2 program. Assessment of this treatment's success largely depends on determination of the expression of the FIX Padua transgene, which is however hampered by the lack of an antibody that distinguishes between wild-type FIX and FIX Padua.

Aims: To generate antibodies that specifically recognize FIX Padua without cross-reactivity to wild-type FIX. Such material should allow assays to be developed that unambiguously detect FIX Padua in clinical samples.

Methods: A phage display method was used to select specific FIX Padua binders. The phage library was screened with a linear and a structural peptide that enclosed the single amino acid substitution at position 338, as well as with full-length recombinant FIX Padua. Three rounds of panning, with and without competition with wild-type FIX sequences, allowed several binders to be identified. BIACORE and ELISA experiments were performed to determine the specificity and affinity of the antibodies obtained.

Results: Various antibodies were initially identified from the different phage display panning routes. However, unique and specific FIX Padua binding was confirmed for one candidate from the linear peptide route (AbD24742.1) only. The selected lead candidate had a detection limit of ~3 ng/mL plasma and showed no cross-reactivity to wild-type FIX even at highly elevated (>50 μg/mL) concentrations.

Conclusions: We developed a highly specific anti-FIX Padua antibody that can be used to develop clinical assays to selectively distinguish between wild-type FIX and FIX Padua antigen levels.

FVIII22

Development and application of a FIX-specific immunoassay to monitor hemophilia B gene therapy

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Background: Gene therapy holds great promise as a future treatment option for hemophilia. In the BAX 335 clinical phase 1/2 trial, an AAV2/8 viral vector is used to express FIX Padua (FIXp), a hyperfunctional variant of FIX with a single amino acid exchange (R338L), in subjects with severe hemophilia B. Specific detection of the transgene product is crucial for assessing the success of the therapy, but challenging for patients with FIX cross-reactive material (CRM+).

Aims: To develop a FIXp-specific ELISA and use this assay to measure FIXp expressed in plasma from hemophilia B patients after treatment with BAX 335.

Methods: The Fab fragment of a newly developed FIXp-specific binding antibody was coated to 96-well microplates at 2 μg/mL using standard conditions. A biotinylated polyclonal sheep anti-human FIX IgG and streptavidin peroxidase were used as detection system. The assay was calibrated by generating a six-point calibration curve with a FIXp preparation, covering a FIXp concentration range of 27.1 to 0.85 ng/mL. Patient samples were diluted with HEPES/NaCl buffer containing 5 mg/mL bovine serum albumin, 10 mM benzamidine, 10 mM $CaCl_2$, and 0.05% Tween 20.

Results: Normal human plasma and purified human FIX showed no signals in the FIXp-specific ELISA. Accurate calibration curves were obtained. FIXp spiked to 1/10-diluted normal human plasma showed acceptable recoveries with dilution response curves parallel to that obtained for the assay standard in buffer. Importantly, analysis of samples of six patients treated with BAX 335 demonstrated highly similar FIXp protein and FIX activity curves over time, and the samples of CRM+ patients showed no increased signals for FIXp protein compared with CRM- patients, indicating the specificity of the assay.

Conclusions: The FIXp-specific ELISA allows additional monitoring of treatment outcome via measurement of the FIXp protein. First data demonstrate the feasibility of this approach.

FVIII24

Benefit of the study of *F8* gene transcription by *in vitro*, *in vivo* and *in silico* analysis in hemophilia A

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Background: Hemophilia A (HA) is a monogenic disease due to *F8* gene alteration. More than 2000 mutations are described, but a number of alleles are yet unidentified.

Aims: Unknown and silent mutations are identified in patients or in women with low levels of FVIII: C, sometimes in an emergency

context of male fetus pregnancy, making genetic counseling difficult. To address these problems we set up a strategy.

Methods: This approach follows a flowchart that combines mRNA study, minigene test coupled with site-specific mutagenesis and *in silico* study using bioinformatics tools. We applied to 18 patients: 10 without identified mutation and 8 with silent (3), new (2), intronic (3) variations. Of these, 6/8 are women with coagulation factor suggestive of carrier, of which 4/6 are pregnant of a male fetus at risk of hemophilia.

Results: Abnormal transcript is identified in 14/18 patients. We identify i)larger RNAs due to inclusion of part of intronic region in 5: intron 1 (3) and intron 18 (2); ii)shorter RNA in 4: 3 are exon 2 deleted and carry a silent variation, 1 is exon 6 deleted. Genomic analysis of the deep intronic regions surrounding deleted exons and included regions allows identifying the mechanism responsible for abnormal splicing. Meanwhile, qualitative and quantitative minigene approach in 5 patients show abnormal transcripts associated with persistence of the full length transcript consistent with residual *F8* mRNA that could explain or be in favor of moderate or mild form of the disease.

Conclusions: These results validate our strategy for the study of presumptive splice mutations and report unanticipated defects in splicing. Such assays improve the genotype-phenotype correlations. This strategy demonstrates its efficiency since it enables to conclude 75% of cases and to answer in difficult situations of women at risk for whom genetic counseling was difficult because of uncertain diagnosis. However its use in routine diagnostic in time frame consistent with emergency situations is still a challenge.

FVIII25

Method-related potency issues of modified Factor VIII products

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Background: New approaches to Haemophilia A replacement therapy include chemical and genetic modifications to the factor VIII (FVIII) molecule to increase post-infusion stability or plasma half-life. Potency labelling is problematic as structural modifications lead to method-related potency discrepancies and labelling of these products in International Units (IU) requires valid assays relative to the WHO 8th International Standard (IS) FVIII Concentrate.

Aims: Results from a single laboratory are reported for the potency measurement, relative to the WHO IS, of five modified recombinant FVIII concentrate products (PEGylated x3, single chain and Fc fusion).

Methods: Assays were performed on the ACL TOP 500 analyser using four chromogenic kits and one-stage clotting assays (OSCA) with eleven APTT reagents. Multiple dilutions of the products and standard allowed comparison of dose-response relationships by parallel line analysis.

Results: Products were independently analysed for linearity and < 2% of analyses (not product specific) were excluded for non-linearity or variability. Chromogenic and OSCA assays for all products fulfilled the criteria for parallelism with dose-response slopes within 80 - 125% of the WHO IS slope. Tukey's multiple comparison test for the OSCA indicated significant differences in the potencies of some modified products when different APTT reagents were used with an extreme potency range of 50 fold for one product. There was good agreement between the four chromogenic kits for all products with a maximum discrepancy of 1.1 fold (see Table).

Table Modified Products: GeoMean and fold values.

	One-stage clotting assay		Chromogenic Assay	
Modified Product	GeoMean range (IU/ml)	Fold difference (high/low)	GeoMean range (IU/ml)	Fold difference (high/low)
A	3.04–7.11	2.3	9.36–9.63	1.0
B	5.46–8.95	1.6	8.21–9.33	1.1
C	6.75–14.79	2.2	9.89–10.59	1.1
D	2.11–3.61	1.7	5.72–6.43	1.1
E	**0.28–14.08**	**50.3**	8.68–9.67	1.1

Conclusions: Modified products can be assayed relative to the WHO IS and potency labelling in IU is possible. However, the measured IU potency can vary widely depending on the method used and it is crucial for manufacturers and regulators to agree on the labelling method to ensure global product consistency.

FVIII26

Individualizing hemophilia prophylaxis using thromboelastography (HIP TEG)

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Background: Most patients with severe hemophilia receive prophylaxis aimed at preventing bleeding events and their sequelae. Dosing derived from cohort studies is a weight-based multi-dose per week approach and is usually not PK(Pharmacokinetics)-based. Personalized medicine has increasingly become an aim in disease management with the goal of providing patients the greatest benefit with the least risk. When attempted, individualized dosing is based on factor levels, yet this approach ignores other components of the coagulation system that vary between individuals. Global hemostasis assays have been studied in hemophilia patients, but so far these tests have not been used to guide prophylactic dosing regimens.

Aims: The purpose of this study is to utilize thromboelastography to individualize prophylaxis regimens in patients with severe hemophilia A.

Table Study summary for modified prophylaxis subjects. (Abstract FVIII26)

Subject ID	Age	Factor Brand	Units	Previous Prophylaxis Regimen	Study Infusion Schedule	Spontaneous Bleeds During the Study	Completed Study	Kept Study Infusion Regimen
HT1-03	12	Helixate	2,000	3x/week	q72 hrs	2	Yes	Yes
HT1-04	14	Helixate	2,000	3x/week	q96 hrs	0	Yes	Yes
HT1-06	13	Xyntha	3,000	3x/week	q72 hrs	3	WD	-
HT1-07	17	Advate	3,000	3x/week	q72 hrs	0	Yes	Yes
HT1-10	5	Advate	800	2x/week	q72 hrs	0	Yes	Yes
HT1-13	12	Advate	2,400	QOD	q96 hrs	1	Ongoing	-
HT1-14	8	Helixate	1,800	QOD	q72 hrs	0	Ongoing	-

Methods: Subjects on prophylaxis who signed informed consent to participate in an IRB-approved institutionally-funded study were enrolled. All subjects were on standard half-life rFVIII concentrates. After appropriate washout, each subject had PK analyses (pre-dose, peak and q24 h until they reached a predetermined endpoint—see below) including measurement of FVIII activity and kaolin-activated TEG. To determine the frequency of infusions, a formula based on R time (TEG) was developed such that when each subject's R time was within 20% of their baseline (signifying insufficient clotting activity), they were required to redose. Patients whose prophylaxis regimen was extended beyond their previously prescribed regimen were followed for 6 months to monitor for bleeding events.
Results: Of 14 subjects enrolled so far, regimen has been extended for 7. Four of these have completed the study and elected to continue on the TEG-guided extended regimen. See Table for data including bleeding.

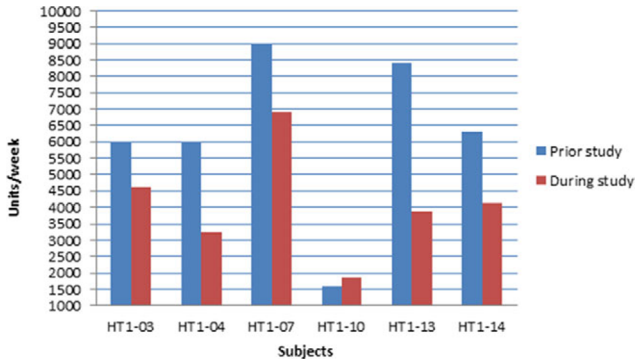

Figure Factor consumption pre- & post-regimen adjustment.

Conclusions: Early results suggest that TEG is a promising tool which can be used to bring a personalized approach to prophylactic factor therapy in hemophilia.

FVIII27

The frequency and possible reasons of hyperhomocysteinemia in patients with severe haemophilia from North-Western Russia

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Background: Severe haemophilia (SH) is often complicated by joint(s) destruction caused by chronic arthropathy due to recurrent haemorrhagic events and activation of such biological mechanisms as oxidative stress and inflammation. The frequency of increased homocysteine (HCy) plasma level, or hyperhomocysteinemia (HHCy), in patients with SH from North-Western Russia (NWR), its possible reasons and role in development of arthropathy are not well known.
Aims: To assess the frequency of HHCy and its possible reasons in patients with SH from NWR.
Methods: We studied 22 men with severe haemophilia A or B (19 and 3 patients, respectively). Osteoarthritis of large joint(s) was detected in each patient, with the rate of recurrent haemorrhagic events in joint(s) from 6 to 13 per year. Standard ELISA assays to measure HCy plasma level and IgG to hepatitis C virus (HCV) were used. Polymorphism C677T in the methylenetetrahydrofolate reductase gene (MTHFR) was studied by PCR and subsequent restriction analysis. Fisher's exact test was used to assess statistical differences between the groups by calculating odds ratios (OR) with their 95% confidence intervals (CI) and p-value.
Results: Hyperhomocysteinemia (HCy level >13.4 μmol/l) was observed in 8 (36.4%) patients. This rate was more than 4-fold increased when compared to frequency of HHCy (8.8%) in healthy persons from NWR (OR=5.9, 95% CI: 2.2–15.5, p=0.0009). Seropositivity for HCV was detected in 75.0% haemophiliacs with HHCy compared to 57.1% in patients with normal HCy level (OR=2.3, 95% CI: 0.3–15.3, p=0.65). The presence of MTHFR C677T allele was found in genotype of 6 (75.0%) patients with HHCy and in 42.9% of other patients (OR=4.0, 95% CI: 0.6–27.3, p=0.2).
Conclusions: We have found high frequency of HHCy in patients with SH. Both genetic (MTHFR polymorphism) and acquired (HCV infection) factors could participate in development of HHCy in this group. Further studies are needed to confirm our data and identify the role of HHCy in development of arthropathy in SH patients.

FVIII28

Product-specific calibration standards do not correct one-stage clotting assay discrepancies for modified recombinant FIX molecules with reagents that significantly over- or under-recover labeled potency

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Background: To prolong the half-life of FIX for factor replacement therapy, modifications such as glycoPEGylation, albumin and Fc fusions have been developed. Varying degrees of reagent specific assay discrepancies related to potency assignment were previously reported for the modified recombinant FIX (rFIX) molecules, with geometric coefficient of variation across multiple labs and reagents reported to be 22.5%, 56.9%, and 260.5% for Alprolix, rIX-FP, and N9-GP, respectively. Multiple methods have been proposed to correct assay discrepancies including the use of product-specific standards.
Aims: To evaluate whether a product-specific standard corrects one-stage clotting (OS) assay discrepancies for modified rFIX molecules with the largest reagent-specific under- or over-recoveries.
Methods: Both glycoPEGgylated FIX (GP-FIX) and FIX albumin fusion protein (FIX-alb) were generated based on INN sequences and published methods. OS assays were performed against WHO FIX concentrate standard for all 3 proteins, and a self-standard assessed for outlier protein/reagent combinations.

Table 1 Activity recovery (%) with different APTT reagents relative to reported potency assignment reagent (*) -against WHO FIX concentrate standard. (Abstract FVIII28)

	Actin FSL	Synth ASil	PTT-A	C.K. Prest	Pathromtin SL	Synth AFax	Actin	Actin FS	APTT-SPActin
FIX-alb	63.9%	70.1%	69.8%	47.6%	100% (*)	ND	ND	ND	ND
GP-FIX	41.7%	30.6%	ND	ND	>1000%	100% (*)	84.8%	24.7%	619.9%
Alprolix	104.1%	85.1%	77.8%	61.3%	66.3%	120.7%	100% (*)	110.6%	79.1%

Results: OS activities for each modified rFIX were normalized to the result of the reported potency assignment reagent of its product counterpart (Table 1). These results were consistent with an NIBSC comparative study on FIX potencies and published data.
When plasma samples spiked with GP-FIX were assayed against a self-standard instead of the WHO standard, recovery of FIX activity below 0.10 IU/ml was not accurate with Actin FS; the clotting times for the entire standard curve were prolonged and the lower end of the curve indistinguishable from background, resulting in loss of sensitivity. Conversely, accuracy was lost at the high end of the standard curve for GP-FIX with Pathromtin SL.
Conclusions: For certain modified rFIX molecule and APTT reagent combinations that significantly under- or over-recovered, applying a

product-specific standard compromised assay performance and thus failed to fully resolve the assay discrepancy issues.

FVIII29

Bleeding scores in hemophilia: are they useful in predicting severity and clinical outcomes

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Background: Hemophilia is an inherited severe bleeding disorder. The ISTH-BAT is a valuable research tool that is applicable to clinical practice and scores patients' bleeding symptoms from 0 to 4. ISTH-BAT has the potential to improve diagnostic accuracy, avoid unwanted laboratory testing, predict the risk of bleeding, describe symptom severity and inform treatment.
Aims: To assess the utility of ISTH-BAT in our hemophilic population, compare the bleeding score (BS) in adult and pediatric groups and investigate its association with plasma factor levels.
Methods: This is an observational analytical study. The ISTH-BAT was used to calculate bleeding scores (BS) in a group of hemophilic patients and healthy controls. Ethics approval and informed consents were secured prior to the study.
Results: A total of 71 patients, (48 hemophilia A; FVIII deficiency and 23 hemophilia B; FIX deficiency) and 30 controls were analyzed using the ISTH BAT. Mean age and mean BAT score in hemophilia A were 15.8±10.1 and 11.5 ±6.52 while in hemophilia B were 20.9±10.5 and 7.9±5.4 respectively. BS were significantly higher in hemophilia A and B patients as compared to controls ($P < 0.05$). ANOVA revealed BS were significantly different among the mild, moderate and severe hemophilia A in both adult and pediatric patients but there was no difference in Hemophilia B patients. BAT scores were linked to hematomas; minor wound bleeding in hemophilia A, B patients in the pediatric group while more linked with epistaxis, umbilical cord bleeding and circumcision in the adult group.
Conclusions: Our data revealed that the ISTH BAT can help diagnose the bleeding condition in hemophilia A and B patients and can be considered a predictor for the bleeding risk or severity. This will ultimately improve the clinical management of patients.

FVIII31

Thrombin generation does not correlate with disease severity but may be predictive of response to treatment in patients with severe hemophilia A

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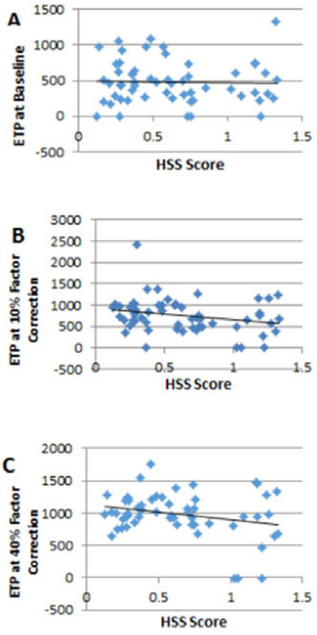
Background: Patients with hemophilia A (HA) have variable bleeding phenotypes despite factor VIII (fVIII) levels of < 1%. We have shown that bleeding severity as assessed by the Hemophilia Severity Score (HSS) did not correlate with endogenous thrombin potential (ETP) at baseline factor levels. We hypothesized that understanding ETP at 10% and 40% fVIII correction levels may better reflect the effects of treatment.
Aims: To evaluate ETP with HSS at baseline, 10% and 40% fVIII correction levels in patients with severe HA without inhibitors. Additionally we aimed to determine if the baseline ETP was predictive of response at spiked levels.
Methods: Baseline plasma samples and HSS scores were obtained from patients with severe HA as part of an IRB approved study. Thrombin generation was evaluated using a standard calibrated automated thrombogram (CAT). Full-length recombinant fVIII was added to patient plasma for final fVIII concentrations of 0.1 U/mL and 0.4 U/mL.
Results: Samples from 57 patients with severe HA without inhibitors were evaluated. CAT mean lag times were 6.37 min (SD 6.36), mean ETPs were 808.87 nM/minute (SD 369.64) and mean peak thrombin levels were 87.45 nM (SD 62.87). Results were divided into quartiles for ETP at baseline, 10% and 40%; 64.9% of patients remained in the same quartiles at 10% or 40% correction. Using linear regression analysis we correlated HSS with ETPs at baseline (P=0.79, R^2=0.001), 10% (P=0.05, R^2=0.06) and 40% (P=0.06, R^2=0.06).

Figure 1: Endogenous Thrombin Potential (ETP) at Baseline (A) and 10% (B) and 40% (C) Factor Correction Levels in Severe Hemophilia Patients without Inhibitors.



These results show no significant correlation of ETP at baseline with HSS but a trend towards significance at 10% and 40% levels.
Conclusions: Our results indicate that ETP does not correlate with the HSS in patients with severe HA at baseline but shows a trend towards significance at 10% and 40%. Baseline ETPs appear to be predictive of response at 10% and 40% fVIII correction levels. Given that the standard of care for patients today is prophylaxis, ETP at different potential trough fVIII levels may be useful in guiding treatment decisions.

FVIII32

Prophylaxis with factor-eight-inhibitor-by-passing-activity 3 days a week results in safe and effective haemostasis in children with haemophilia a with inhibitors

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Background: Inhibitors to Factor VIII replacement therapy occur in 35% of severe Haemophilia A children. Current recommendations are to use prophylaxis with Factor-Eight-Inhibitor-By-Passing-Activity (FEIBA) every other day in patients who have failed conventional immune tolerance therapy (ITI), or in those patients unable to receive ITI. In patients on ITI, recommended FEIBA prophylaxis is twice a day. Drawbacks to this approach include high treatment burden, frequent central line use and high cost to the patient and medical system.
Aims: To achieve a balance between treatment burden and cost with maintaining good prevention of bleeding by using FEIBA prophylaxis only 3 times a week whether the patient is receiving ITI or not.
Methods: The records of 10 children with severe Haemophilia A with inhibitors were reviewed. All patients received FEIBA prophylaxis 75–100 units/kg 3 days per week until inhibitor titers decreased enough to start ITI.
Results: Median time on FEIBA prophylaxis was 16.5 months (mean 30, range 1–84). Seven patients experienced no major bleeding while on prophylaxis. Three patients had repeated spontaneous bleeding episodes despite FEIBA prophylaxis that only resolved with a switch to recombinant factor VIIa (1 patient) or starting ITI (2 patients). Six patients had a gradual decrease in inhibitor titer during prophylaxis. A transient rise in inhibitor titer occurred on 4 occasions in 3 patients: 2 were associated with a central line infection, 1 spontaneous, 1 due to ITI failure. In all 4 episodes, the titer subsequently decreased. There were no thrombotic episodes. One patient stopped prophylaxis due to anaphylaxis. All patients were able to start ITI and eventually stop FEIBA prophylaxis (range 0–12 months).
Conclusions: Reduced frequency FEIBA prophylaxis did not result in increased spontaneous bleeding or significant anamnesis. This regimen is safe, effective and can be considered in patients unable to receive ITI or in ITI patients who need additional therapy to prevent spontaneous bleeding.

FVIII33

Results from a world-wide field study of FVIII activity assay variability of BAX 855, the PEGlylated form of rFVIII ADVATE, in clinical hemostasis laboratories

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Background: Discrepancies were reported for BDD and some modified longer-acting FVIII products when one-stage clotting assays were used for FVIII activity analysis.
Aims: A multi-national collaborative field study among clinical and hemostasis laboratories to analyze plasma from patients with hemophilia A spiked in vitro with BAX 855 (ADYNOVATE/ADYNOVI) and ADVATE was performed.
Methods: FVIII was spiked at 0.80, 0.20 and 0.05 IU/mL based on labeled potencies. Samples were blinded and sent to participating sites. Laboratories analyzed samples with their routine FVIII assay and reported results.
Results: 35 data sets were reported. All laboratories were using a one-stage clotting assays (OSCA) method, 11 also used chromogenic assays. No single laboratory performed a chromogenic assay only. At the highest FVIII concentration, the mean in vitro recovery relative to expected, using OSCA was 101% for ADYNOVATE and 114% for ADVATE. With decreasing FVIII concentrations, recoveries showed a trend to overestimation, however on average, recoveries were comparable for BAX 855 and ADVATE. Inter-laboratory variability increased with decreasing FVIII concentrations but was very similar for both products. For chromogenic FVIII activity assays, in vitro recoveries across all concentrations were comparable for BAX 855 and ADVATE. Variation increased with lower FVIII concentrations. At the lowest concentration it even exceeded variability of OSCA.
Conclusions: All types of OSCA and chromogenic assays can be used for FVIII activity measurement of BAX 855 and ADVATE in hemophilic plasma, resulting in similar accuracy and precision for both products. In general, at very low FVIII concentrations, higher variability should be expected with chromogenic assays. The results indicate no need for a product specific standard for BAX 855, similar to widely used and well established full-length rFVIII product ADVATE.

FVIII34

Reagents causing N9-GP underestimation in one-stage FIX clot method inhibit FXIa-catalysed FIX activation and discriminate between N9-GP and FIX in plasma independently of contact activation and phospholipid

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Background: Some aPTT reagents, with no obvious common denominator, are reported to give 30–40% recovery of glycoPEGylated recombinant FIX (N9-GP) in one-stage (OS) FIX clot method compared with that of native FIX.
Aims: To investigate the cause of N9-GP underestimation.
Methods: Experiments mimicking the entire course of the OS method or the clotting phase (FXIa added to omit contact phase) were performed with and without plasma, aPTT reagent and added defined phospholipid (PL). Activation of N9-GP and recombinant FIX (rFIX) during the clotting phase was measured as FIXa activity in quenched withdrawn samples using the Rox FIX-A kit. FXIa-catalysed amidolysis and cleavage of N9-GP/rFIX were measured with and without aPTT reagent present, the latter monitored by SDS-PAGE (protein/PEG staining).
Results: Three reagents (SynthASil, Actin FS and CK Prest) which underestimate N9-GP in OS FIX clot method also did so in our systems, even when the contact phase was omitted and when PL was added. The reagents reduced the activation rate of both N9-GP and rFIX in the presence of plasma and calcium. The decrease in N9-GP activation was greater, which would explain the underestimation of N9-GP by clot method. SynthAFax, which gives the expected N9-GP recovery, neither differentiated between N9-GP and rFIX nor inhibited activation. Omission of plasma led to severely impaired generation of FIXa from both N9-GP and rFIX with the underestimating reagents, and FXIa amidolytic activity was inhibited by ~55%. Using SynthASil and SDS-PAGE analysis, we confirmed reduced rates of FXIa-catalysed cleavage, via a known pathway, of N9-GP and rFIX.
Conclusions: In the presence of plasma, the underestimating aPTT reagents impose a general inhibition on FIX activation, most pronounced for N9-GP. The underestimation is revealed during the clotting phase but apparently not caused by the PL. In the absence of plasma, FIX activation is further inhibited, reaching similar levels for N9-GP and rFIX.

FVIII35

Multi-national survey of FVIII activity assay practice

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Background: Historically clinical laboratories used one-stage clotting assays for FVIII activity determination for diagnosis of hemophilia, determination of the phenotypic severity of FVIII deficiency, and to monitor post-infusion FVIII levels to adjust dosing and personalize treatment regimes. Based on literature and feedback from experts it is assumed that the vast majority of laboratories still use one-stage clotting assays while chromogenic assays are rarely used, mainly in addition to one-stage clotting assays to confirm results and/or refine analysis, particularly for diagnosis.

Aims: A multi-national survey of FVIII activity assay practice was performed to investigate current FVIII activity assay preferences. Here we report the results of the survey.

Methods: A questionnaire was sent to 127 laboratories in 25 countries in all geographies. The questionnaire asked for details on assay methods, instruments, assay reagents including FVIII deficient plasma and aPTT reagent, reference standard, frequency of preparation of reference curve, number of different dilutions tested per sample, assay controls, and further details on patient plasma samples testing. Feedback was collected and the answers were evaluated.

Results: 56 laboratories returned the completed questionnaire. From these laboratories, 98% perform and rely on the one-stage clotting assay, and 73% are using the one-stage clotting assay as their sole FVIII activity test system. Only one single laboratory (2%) uses the chromogenic assay only, while 25% have both assays available. From the laboratories performing the one-stage clotting assay as their preferred assay system, the majority of them (58%) use silica based aPTT reagents.

Conclusions: The survey showed that in clinical practice the one stage assay is the currently by far preferred type of FVIII assay used. This confirmed previously published and reported expert opinion. Most frequently silica based aPTT reagents are used.

FVIII36

Effects of haemolysis, icterus and lipaemia on coagulation tests as performed using mechanical viscosity based endpoint detection

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Background: Between 1/3 and ¾ of laboratory errors occur during the preanalytic phase. Haemeolysis, icterus and lipaemia (HIL) may affect haemostasis test results. This can be influenced by the level of interfering substance, the assay principle, and the endpoint detection system used.

Aims: To assess the influence of HIL on the results of PT, aPTT and fibrinogen assay performed with different reagents on a viscosity-based detection analyser.

Methods: Interference of haemolysis was studied using reject haemolysed patient samples matched to non haemolysed replacements and by mechanical cell lysis. PTs were determined with STA®-Neoplastine® R and STA® Neoplastine® CI Plus and APTTs with STA®-PTT Automate, STA®-Cephascreen® and STA®-C.K. Prest®. Fibrinogen reagent was STA®-Liquid Fib. All assays were performed using an STA-Compact Max® analyser (Stago, Asnières/Seine, France).

Results: Spontaneous haemolysis occurring during sample collection and processing had no effect on PT with either reagent. In contrast, mechanically haemolysed cells impacted statistically significantly PT for the highest haemoglobin concentration. For APTTs determined with STA®-Cephascreen® reagent there was no significant difference between results in haemolysed and non haemolysed samples. For the other two reagents studied APTTs were statistically significantly shorter in haemolysed samples compared to matched non haemolysed samples. However, this bias was clinically significant only for STA®- PTT Automate. The APTTs of some haemolysed samples were falsely normal only with STA®- PTT Automate which was more affected than the two others. Fibrinogen results were not affected by either type of haemolysis with the tested reagent. The effects of lipaemia and icterus were not clinically significant whatever the reagent used.

Conclusions: Overall our results confirm that PT and fibrinogen were not clinically significantly affected by HIL. Haemolysed samples for APTT determination should be rejected.

FVIII37

Mutational anaylysis in 18 Pakistani families with Haemophilia B: identification of a novel mutation

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Background: Both gain of function and loss of function mutations have been reported in factor IX gene (F9). The later result in haemophilia B (HB), also called factor IX (FIX) deficiency. Scheme of mutations for the disease has been heterogeneous across the globe. Different mutations may translate into phenotypically identical outcome and vice versa. Spectrum of mutations in FIX gene in local HB population is still unknown, with only one study on board so far.

Aims: The current study was aimed to sort causative mutations in Pakistani families with HB and determine their relationship with corresponding phenotype.

Methods: Mutations were identified in all the patients employing Sanger sequencing technique. Phenotypic analysis comprised base line coagulation profile seconded by FIX activity (FIX: C) and inhibitors assays. Characteristics of the novel mutation were determined by bioinformatics tools.

Results: A total of 16 point mutations, 01 small deletion and 01 splice site mutation were identified. Cumulatively, 16 unique variants were identified including one novel mutation. The novel mutation was substitution point mutation in exon 7 which clinically reflected as moderate FIX deficiency. Homology modeling of the novel mutation revealed abnormality in the protein structure which was in apposition with the clinical phenotype.

Conclusions: It was concluded that spectrum of causative mutations for HB in Pakistani population is heterogeneous. The phenotypic correlation is in agreement to what has previously been sorted for the corresponding mutations in other populations. It is also recorded that a novel point mutation (c.756T>G) in the gene is potentially pathogenic, clinically corresponding to moderate severity of the disease.

FVIII38

Qualification of selected one-stage APTT and chromogenic assays for the post-administration monitoring of nonacog beta pegol

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Background: Nonacog beta pegol (N9-GP) is a glycopegylated recombinant human coagulation factor IX.
Aims: Qualification of N9-GP using selected one-stage APTT and chromogenic assays.
Methods: The *in vitro* recovery of N9-GP was assessed at three independent qualification sites for three FIX activity assay reagents, the STA®-Cephascreen® one-stage clotting reagent, using STA®-Deficient IX plasma and STA®-Unicalibrator from Diagnostica Stago, Inc., the ROX FACTOR IX chromogenic assay from Rossix AB and the BIOPHEN Factor IX chromogenic assay from Hyphen Biomed SAS. Samples containing N9-GP spiked into congenital FIX-deficient plasma at concentrations between 0.05 and 2.5 IU/mL were prepared at Esoterix, Inc. in Englewood, CO, USA and distributed to all three sites for each reagent-instrument system. Testing at each site for each FIX activity reagent-instrument system was performed in accordance with approved site-specific qualification protocols.
Results: For each of the three reagents (in total five different reagent-instrument systems), acceptable intra- and inter-assay accuracy and precision, dilution integrity, reagent robustness and acceptable sample stabilities (i.e. freeze-thaw, short-term and long-term sample stability) were demonstrated with the following limitations: limited upper-reportable-range was observed for STA®-Cephascreen® at one of three qualification sites and suspected low-end sensitivity for the BIOPHEN Factor IX assay was observed at one of the three qualification sites. The impact of these limitations on the respective assay qualifications were considered to be none, because these would be addressed at the local laboratory level by adjusting the reportable range of the assay.
Conclusions: The STA®-Cephascreen® with STA®-Deficient IX on the STA-R Evolution®, as well as the ROX FACTOR IX and the BIOPHEN Factor IX chromogenic assays were considered qualified for the measurement of N9-GP in 3.2% citrated human plasma.

FVIII39

Diagnosis and management of acquired haemophilia - single centre experience

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Background: 29 patients with acquired haemophilia (AH) have been diagnosed and treated in our centre between 2002–2015.
Aims: To present detailed clinical observation concerning cases of AH, which is a rare and life-threatening bleeding disorder.
Methods: 29 patients aged from 23 to 87 years with no personal or family history of bleeding diathesis were examined for sudden onset of severe bleeding symptoms and prolongation of APTT.
Results: Detailed coagulation testing showed decrease of factor VIII level - the average was 4,8% (range: 0–33%) and presence of factor VIII inhibitor - average level was 200 BU (range: 0.8–1600 BU). 86% (25/29) patients developed skin hematomas, muscles were second most common bleeding site (48%, 14/29). Unlikely in congenital haemophilia only one patient bled into joints. None of the patients had intracranial haemorrhage, but 31% (9/29) patients bled severely.
All the patients received immunosuppressive therapy. One patient was treated with corticosteroids only, while all the other patients received corticosteroids combined with cyclophosphamide. Remission was achieved with this treatment alone in 34% (10/29) cases. Patients resistant to induction therapy received intravenous immunoglobulins, cyclosporine-A or anti-CD20. One patient was treated by extracorporeal immunoadsorption as well. Bypassing therapy with rFVIIa or APCC was used in 69% (20/29) patients.
Conclusions: 72% (21/29) patients achieved complete remission defined by normalisation of FVIII level and cessation of bleeding. Average time to remission was 9,6 weeks (range: 2–49 weeks). 21% (6/29) patients died between 4th and 7th week of treatment, but only one of them died of uncontrollable bleeding. Other three patients died of surgical complications. Overall, the treatment of AH was successful in most cases and bypassing agents proved good efficacy to minimize bleeding in the patients.

FVIII40

Turkish experience with low dose of immun tolerance treatment for inhibitor patients

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Background: Inhibitor development is the main complication of FVIII treatment in patients with severe hemophilia-A. Gold standart treatment is immune tolerance treatment (ITT) for eradication of inhibitors. With higher dose ITT, more successful results was reported for patients with high risk factors. However it is not easy to use due to reimbursement limitations.
Aims: Our aim was to show same succeess rate with low dose ITT in Turkish hemophilia pateints with inhibitors.
Methods: With this national study, we have collected data for 21 patients who treated with ITT from 11 hemophilia centers. All patients with severe HA (< 1%) and 19 were HR and 2 were LR patients. All patients were more bleedy and need to use by-passing agents. Mean age was 7 yr (6 mo-32 yr). 19 pateints were used low dose ITT (50 IU/kg 3 times a week). Another three patients were used higher doses (100 IU/kg 3 or 7 days a week). 15 patients were used pd-FVIII others were recombinants.
Results: Seven patients (%33) were eradicated (negative for Bethesda and normal recovery test). Another 2 patents (10%) had negative for inhibitors but recovery was abnormal. Other 12 patients had inhibitör positivity. Six cases (28%) had < 5BU of titrages and continued with FVIII prophylaxis. Totally 8 patients had partial response.. Other 6 patients were not responded. We compared two groups as eradicated and non-eradicated. Eradicated patients had lower age (4.5 yr vs.10yr), lower peak titers (20 BU vs. 93 BU),more shorter duration time (11 mo vs 13 mo) and shorter waiting time (11 mo vs. 4.5 yr). Interestingly in low dose group, 6 out of 7 patients were eradicated whereas in high dose group only one out of three cases were eradicated.
Conclusions: Even with low dose ITT, one third of patients were able to be eradicated. Two third of patients allowed to continue FVIII prophylaxis and no need for by-passing agents. However one third of cases needed by-passing agents for bleeds. We recommend low dose ITT treatment for HR inhibitor patients for developing countries.

FVIII42

Haemophilia A and immune tolerance induction - our first experiences

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Background: Inhibitors affect about 1/3 of people with severe haemophilia A. Most people develop these inhibitors when they are very young children, soon after they receive their first infusions of factor VIII (FVIII) concentrate. In 2 out of 3 of cases the inhibitors disappear on their own, or with treatment, within, on average, 9 months. For the others, the inhibitors persist and are a serious problem. The different treatments for eradication of inhibitors are called immune tolerance induction (ITI). Different protocols of ITI have been found successful. All the therapies include high doses of factor VIII for long periods. These therapies last anywhere up to 36 months. They are successful with 2 out of 3 people with severe haemophilia A.
Aims: The aim of the work is to present the recent literature data about ITI in haemophilia A with inhibitor. Special attention was paid to own three successful ITI procedures using plasma derived FVIII concentrate rich in von Willebrand factor (pdFVIII/vWF) and pulsed i.v. immunoglobulins.
Methods: As ITI we used the modified Bonn protocol with the high doses of pdFVIII/vWF and pulsed intravenous immunoglobulins in three patients with haemophilia A and high titer inhibitor.
Results: Three ITI procedures using plasma derived FVIII concentrate rich in von Willebrand factor (pdFVIII/vWF) and pulsed i.v. immunoglobulins were succesful in patients with congenital haemophilia A and high titer inhibitor.
Conclusions: Several modifications of ITI were described, but none was approved to be superior. However, according to the literature and our own experiences the modified Bonn regimen (high doses of pdFVIII/vWF and pulsed intravenous immunoglobulins) was very effective in patients with congenital haemophilia A and high titer inhibitor with unfavourable prognosis of ITI. The study was supported by projects Vega 1/0168/16, APVV 0222-11 and BioMed Martin, ITMS 26220220187).

FVIII43

N9-GP activity can be measured using select one-stage clot assays and two-stage chromogenic assays in clinical samples from adults/adolescents and children with haemophilia B

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Background: N9-GP (nonacog beta pegol) is a glycoPEGylated recombinant Factor IX (rFIX) with an improved pharmacokinetic (PK) profile compared with conventional rFIX products. One-stage clotting (OS) assays have typically been used to assess FIX activity, but chromogenic substrate (CS) assays are now available. The safety, efficacy and PK of N9-GP have been evaluated in the paradigm™ clinical trial programme in which FIX activity was measured using both the OS and CS assays.
Aims: To compare FIX activity measured using the OS and CS assays in adults/adolescents and children with haemophilia B treated with N9-GP.
Methods: Patients received once-weekly N9-GP prophylaxis for 52 weeks (40 or 10 IU/kg in adults/adolescents [13–70 years; paradigm™2]; 40 IU/kg in children [≤12 years; paradigm™5]). Single-dose and steady state PK were assessed in 17 adults/adolescents and 25 children: pre-dose and at 30 min, 8 h (adults/adolescents only), 24 h, 48 h, 96 h and 168 h after the first dose. FIX activity was measured using OS (SynthAFax) and CS (BIOPHEN) assays. To compare FIX activity levels measured with the two assays, the ratio between each individual result was plotted against the geometric mean of results from the two assays using a Bland-Altman plot.
Results: In all patients, high recovery and trough levels for N9-GP were consistently detected with both OS and CS assays, after single-dose infusion and at steady state. The ratio of FIX activity levels between the OS and CS assays in both adults/adolescents and children was approximately 1. This represented a high correlation in FIX activity over a large range of measurements (0.01–1.11 IU/mL), with no systematic difference between the two assays.
Conclusions: When monitoring N9-GP activity in haemophilia B patients, the OS (with select reagents) and CS assays are comparable.

FVIII44

Factor VIII effect and sensitivity vary depending on trigger in thrombin generation assays with plasma from patients with hemophilia A

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Background: Measuring coagulation function is essential for monitoring hemophilia treatment. The tissue factor (TF)- or extrinsically triggered thrombin generation assay (TGA) is often utilized to monitor global hemostasis in hemophilia A. Sometimes, thrombin generation (TG) is initiated with FXIa through the intrinsic pathway leading to higher FVIII sensitivity. Recently, TGA has also been used to determine FVIII equivalency of FVIII mimetic agents.
Aims: We studied the FVIII effect on TG initiated using different trigger types and concentrations.
Methods: The calibrated automated thrombogram (CAT) assay- based on the fluorogenic substrate Z-G-G-R-AMC- was performed in hemophilia A patient plasma. Recombinant FVIII (3–1000 mU/mL) was titrated and TG triggered with TF (0.4 -20 pM) or FXIa (31 -1000 pM) and 4 μM phospholipids. The CAT parameters lag time, thrombin peak, time to peak, and endogenous thrombin potential (ETP) were analyzed.
Results: Generally, TF-triggered CAT resulted in lower TG in response to FVIII than the FXIa-triggered CAT. Thrombin peak and ETP values increased with rising TF concentrations, whereas FVIII sensitivity was gradually decreased. TG at 1 pM TF was FVIII sensitive to ³ 100 mU/mL FVIII, but almost independent of FVIII at 20 pM TF. The time parameters changed only moderately or not at all depending on FVIII. In the FXIa-triggered CAT, the FVIII sensitivity range shifted depending on the FXIa concentration. For example, the sensitive FVIII range was 10–100 mU/mL and 1–30 mU/mL for 125 and 500 pM FXIa, respectively. An increase in thrombin peak corresponded well with shortened time parameters.
Conclusions: The CAT is a versatile tool to assess the hemostatic effect of FVIII in plasma from hemophilia patients. Depending on trigger,

FVIII sensitivity varies and FVIII displays distinct effects on the CAT parameters. Therefore, trigger type and concentration strongly influences the assay outcome and may lead to diverse FVIII equivalent values when used for assessment of FVIII mimetics.

FVIII45

High agreement rate between ellagic acid-based one stage and chromogenic assays for rFVIIIFc post infusion samples in the A-LONG study

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Background: The one-stage (OS) clotting assay and the chromogenic substrate (CS) assay are the most frequently used assays for treatment monitoring in haemophilia A. Discrepancies between OS and CS occur, and concerns have been expressed regarding the accurate measurement of factor activity of certain Extended Half-Life (EHL) products [Peyvandi 2015]. The European Medicines Agency (EMA) requires CS for potency assignment of FVIII concentrates, however, potency labelling with OS may also be approved provided equivalence can be shown between both methods [EMA Report 2014]. A field study showed the accuracy and reliability of measuring rFVIIIFc in spiked samples of 5, 20 and 80 IU/dL, with good correlation between OS and CS and no reagent specific variability [Sommer 2014].

Aims: To evaluate the agreement rate between OS and CS for rFVIIIFc compared to rFVIII in post-infusion samples from the phase 3 A-LONG study.

Methods: FVIII activity was measured with OS (Siemens BCS-XP analyser; Siemens Actin FSL EA-activator) and CS (Biophen FVIII: C) for both rFVIII (ADVATE®) and rFVIIIFc (Elocta®) in the sequential PK analysis subgroup and for rFVIIIFc in all patients of A-LONG study. OS/CS agreement analysis of post infusion activity values in the range of 1 to 100 IU/dL, and around 5, 20 and 80 IU/dL, was performed and displayed in Bland-Altman plots.

Results: Agreement for activity levelsaround 5 IU/dL is shown in Figure 1. Solid lines represent means; dotted lines represent 95% CI. Similar plots have been obtained for the other FVIII activity values. Overall, the agreement was high and similar for rFVIIIFc and rFVIII at all activity levels.

Conclusions: rFVIIIFc showed a high agreement between OS and CS in rFVIIIFc- post infusion samples, comparable to rFVIII, demonstrating that rFVIIIFc can be reliably measured with both OS and CS at all clinically relevant plasma activity levels.

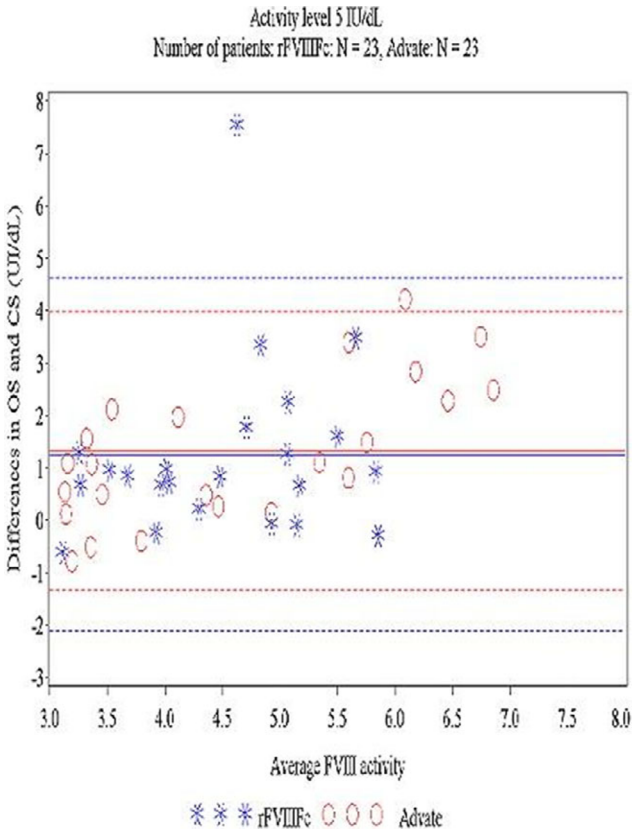

Figure 1 (Abstract FVIII45)

FVIII46

A simple predictive model of thrombin generation for the individualization of hemophilic treatment

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Background: Hemophilia A and B are genetic diseases where respectively coagulation factors VIII and IX are deficient, which leads to a bleeding tendency. Individual management of hemophilia should rely on infusion of a specific quantity of the missing factor, allowing normalization of the patient's thrombin generation (TG) potential. The target level of the missing factor should be patient-dependent. A numerical predictive tool, capable of adequately modeling TG, could help to determine this patient-specific level. However, existing kinetic models, taking into account the numerous coagulation factors, are very complex and fail to accurately predict the TG of hemophilic patients.

Aims: To develop a reliable and simple model capable of predicting patient-specific TG.

Methods: Experimental data were obtained by measuring TG in Platelet-Poor Plasma (PPP) from 40 hemophilic A (HA) and 32 hemophilic B (HB) patients, using the CAT system, with 1 pM tissue factor, and by measuring clotting factors with standard chronometric or colorimetric methods. We built a simplified numerical model based on the experimental data, with clotting factor levels as the inputs and TG as the output. This model includes 11 parameters describing the reaction kinetics between the various factors. We searched for the kinetic parameters that rendered the simulated TG curves identical to those measured using genetic algorithm optimization. Agreement between the measured and model-generated curves was evaluated by linear regression analysis with calculation of R^2.

Results: Comparison between the predicted and measured TG curves showed good agreement between the model-simulated and experimental data ($R^2 = 0.95$) by adjustment of only 3 out of the 11 parameters.
Conclusions: This is the first simple model dedicated to the prediction of TG in hemophilic patients. Future work will focus on refining the model ability to predict the TG curve evolution with treatment. This approach provides a new prospect for the hemophilic treatment individualization.

FVIII47

Effectiveness of heat inactivation in removing residual FVIII interference in the FVIII Nijmegen bethesda assay

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Background: In an effort to avoid reporting false negative results, heat inactivation (e.g. pre-incubation of patient samples at 56–58°C for 30–90 min) is commonly used to remove residual endogenous or exogenous FVIII prior to performing FVIII Nijmegen Bethesda analysis. To date, only limited data exist demonstrating the effectiveness of such heat pre-treatment on the reported inhibitor titer.
Aims: To determine the effectiveness of heat inactivation, on measured low level FVIII Nijmegen Bethesda titers, in FVIII inhibitor positive samples spiked with varying amounts of recombinant and plasma-derived FVIII replacement products.
Methods: Low level FVIII inhibitor samples (i.e. inhibitor positive patient samples, as well as, sheep anti-human FVIII inhibitor samples) containing various amounts of FVIII replacement product were prepared and tested in the Nijmegen Bethesda assay both in the presence and absence of heat inactivation (i.e. 58°C for 90 min).
Results: The FVIII level at which heat inactivation was effective, varied depending on the FVIII replacement product tested. For most products, a low level inhibitor sample (i.e. 1 BU) spiked with FVIII up to 10 IU/dL, when tested in the presence of heat inactivation, remained positive (≥ 0.6 BU). In the absence of heat inactivation, most samples provided negative inhibitor results. FVIII product specific responses were obtained with added FVIII levels > 10 IU/dL, with false negative inhibitor titers observed for many of the replacement products tested. Low level inhibitor titers, even in samples containing no added FVIII, were higher in the presence compared to the absence of heat inactivation.
Conclusions: The effectiveness of heat inactivation in removing interference from residual exogenous FVIIII activity may be product specific. Laboratories performing inhibitor testing post-replacement therapy should consider verifying the limits of their heat inactivation procedure prior to reporting Nijmegen Bethesda results.

FVIII48

Haemolysis effects on clotting factor and thrombotic assay results using matched haemolysed and non-haemolysed patient samples and two artificial haemolysis mechanisms

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Background: 'Spurious haemolysis' created during poor venepuncture account for around 40% of coagulation sample rejections. Scientific literature regarding effects of haemolysis on non-routine coagulation assays are sparse with results mainly produced using artificially haemolysed plasma and not paired patient plasma samples (haemolysed reject and a clear plasma replacement).
Aims: To show effects of spurious haemolysis on specialist coagulation assays using paired-patient samples. To ascertain if artificially haemolysed methods used to assign analyser haemolysis flags are representative of spurious haemolysis.
Methods: 44 anonymised paired patient samples were collected, 29 analysed for one-stage factors; II, V, VII, X, VIII, IX and XI, and Siemens INNOVANCE® von Willebrand Factor Activity (vWF:Act) using the Sysmex CS5100 and 36 pairs for antithrombin (AT), protein C (PC), protein S (PS) and activated protein C resistance (APCV) on the Werfern ACL Top (21 pairs were used in both thrombotic and factor assays). Mechanical and freeze fracture haemolysis were prepared using 10 consenting normal donors, 5 to analyse thrombotic and 6 for factor assays.
Results:
Table 1 Paired Patient Samples Clinically Affected.

Sample Identification	Assay	Non-haemolysed plasma result (normal or abnormal)	Haemolysed plasma result (normal or abnormal)	Analyser Haemolysis Flag (1–5)	Plasma Haemoglobin g/L
23	AT	NORM	ABNORM	2	2.1
29	AT	ABNORM	NORM	*	11.0
42	PS	NORM	ABNORM	2	1.3
12	PS	ABNORM	NORM	1	0.3
52	PS	ABNORM	NORM	2	1.1
50	FVII	ABNORM	NORM	5	5.8
50	FIX	ABNORM	NORM	5	5.8
17	FIX	NORM	ABNORM	1	0.3

Patient assays demonstrating statistical significance were PS, FVII vWF:Act and PC (denoting possible activation of clotting via extrinsic pathway through FVIIa-TF complex and platelet activation). Mechanical haemolysis showed significant.
Conclusions: Testing haemolysed patient samples would have resulted in 7 of the 44 patients being clinically misdiagnosed, 4 of which had haemoglobin concentrations of 1.3 g/L or less. In addition all individual patient assays performed showed unpredictable gains and losses of activity. Assessment regarding haemolysis limitations should include patient sample analysis in both laboratory and manufacturer settings.

FVIII49

Application of rox Factor IX on the ACL TOP 700

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Background: Rox Factor IX is a CE marked chromogenic kit for determination of Factor IX (FIX) activity in plasma, comprising FIX activation by FXIa with simultaneous activation of FX in the presence of FVIII, calcium ions and phospholipids. The amount of FXa formed is related to the FIX activity and is determined from the hydrolysis of a chromogenic FXa substrate. The kit provides a manual method but in most laboratories analyses are performed on automated instruments.
Aims: The aim was to evaluate the performance of an application for use of Rox Factor IX on the ACL TOP 700.
Methods: The performance of the application has been determined by use of EU and CLSI Guideline protocols. The same assay conditions are utilized as for the manual method but in order to compensate for the reagent dead volume of the instrument, all sample and reagent volumes are 20% reduced. Sample FIX activity is derived from a log response/log FIX dose calibration curve using quadratic curve fit. Plasma sample dilution is in general 1:80 but using dilution 1:20 for measuring low FIX activities (< 0.02 IU/mL) and dilution 1:160 for measuring high FIX activities (>1 IU/mL).
Results: The linear measuring range was 0.005 - 2 IU/mL. LOD and LOQ were 0.0025 and 0.005 IU/mL, respectively. The within-assay imprecision was ≤ 7% at tested levels 0.01, 0.2 and 0.9 IU/mL and the between-assay imprecision was 10% at 0.01 IU/mL and 9% at 0.9 IU/mL. The within-assay accuracy bias was 10% at 0.01 IU/mL and within 3% at 0.2 and 0.9 IU/mL. The between-assay accuracy bias was within 3% at 0.01 and 0.9 IU/mL. The method correlated well to a one-stage method, slope = 1.02, r = 0.994 (n = 40). No carry-over was

identified from repeated analyses of samples containing 0.01 and 0.2 IU/mL FIX activity. The on-board stability of the reagents was determined to 48 h.
Conclusions: In summary, the results demonstrate the suitability of the application of Rox Factor IX on ACL TOP 700 for determination of FIX activity in plasma such as for diagnosis of Hemophilia B.

FVIII50

The utility of ISTH BAT in assessing the bleeding phenotype in patients with FV deficiency

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Background: Congenital factor V deficiency (FVD) is a rare autosomal recessive bleeding disorder that accounts for ~8% of all rare bleeding disorders. Clinical manifestations of FVD are diverse, ranging from being asymptomatic to life threatening bleeding conditions, and plasma levels have limited correlation with the severity of bleeding. So far there has been no reports on the use of ISTH BAT in rare coagulation factor deficiencies.
Aims: To investigate the role of ISTH BAT in assessing the bleeding symptoms and severity of the disease in a group of patients diagnosed with FVD.
Methods: A total of 10 patients; 9 with single FVD (8 severe and 1 mild) and 1 with combined FV and FVIII and 16 healthy controls were evaluated.
Results: The mean age of patients was 17, and 50% were females with consanguinity present in 80% of patients. Plasma FV levels ranged between 0.2–1.2% in the severe cases and bleeding symptoms included bruising, dental and GI bleeding, menorrhagia, postsurgical, haemarthrosis and intracranial bleeding. ISTH BAT scores ranged between 4 and 17 with a median of 7. Bleeding scores (BS) were significantly higher in FVD patients compared to that of controls ($P<0.0001$). BS did not correlate significantly with plasma FV levels ($P=0.96$) or with patients' age ($P=0.86$).
Conclusions: Standardized bleeding score via ISTH BAT can help diagnose the bleeding condition in FVD but cannot assess severity or predict the bleeding risk. The small sample size in the different severity groups and the combined FV and FVIII prevented subgroup analysis. Prospective multicenter studies with larger numbers of patients are recommended. Additionally, other more useful tools are required for assessing severity and predicting the bleeding risk.

FVIII52

Application of global coagulation assays in hemophilia B patients to evaluate the hemostatic potential in baseline conditions and after FIX concentrate administration

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Background: Hemophilia B (HB) patients (pts) show a variable bleeding tendency. This heterogeneity is poorly understood.
Aims: Application of global coagulation assays in HB pts, in baseline conditions and after rFIX concentrate infusion.
Methods: Bleeding phenotype (BP) was defined severe or mild considering the annual bleeding rate, orthopedic Gilbert score and mean annual consumption of FIX concentrate from the last 5 years. Thromboelastography (TEG) and Calibrated Automated Thrombography (CAT) were performed before and after rFIX concentrate infusion.
Results: 5 HB pts were enrolled: 2 moderate (FIX:C 4.5% and 4.3% respectively, both mild BP) and 3 severe (FIX:C< 1%, 1 mild BP, 2 severe BP). As regard as TEG, in baseline conditions, *R* (n.v.:4.3–6.3min) was increased inversely to the FIX:C levels (moderate pts *R*: 7 and 10.7 min; severe pts *R*: 16.8, 25.9, 22.7 min, respectively). In moderate pts, *R* was the only altered parameter. In severe pts, *a-Angle* and *Maximum Amplitude* were always altered. Lysis parameters (Ly30/60%) were increased in 1 severe patient (pt). After FIX concentrate infusion, all TEG parameters were normalized. Concerning CAT, in baseline conditions, *lag time* (n.v.:4.3–9.4min) was increased in 2 severe pts with severe BP (10 and 14 min, respectively) while was normal in 2 moderate (4.7 and 5.8 min) and in 1 severe pt with mild BP (5.1 min). Regarding *ETP, Peak, time to-Peak,* we found alterations in all pts. After FIX concentrate infusion, we observed a normalization just for *time to Peak* in moderate pts and in 1 severe pt with mild BP.
Conclusions: In 5 pts, TEG parameters seem not to be informative for BP, because related to the FIX:C level. Regarding CAT, the *Lag time* could be informative for BP, because in baseline conditions is normal in the 3 pts with mild BP. Replacement therapy determines always at least an improvement on TEG/CAT parameters, related to an increase of FIX:C levels. These data need to be confirmed on a larger population.

FVIII53

The development of arterial thrombosis in hemophilia A - unusual coincidence? case report and review of the literature

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Background: Hemophilia A is defined as X-linked recessive bleeding disorder associated with defective synthesis of factor VIII (FVIII). However, it does not prevent patients from the deevelopment of thrombotic episodes. Cardiovascular complications develop even earlier and significantly more often in patients with hemophilia A than in *controls. Moreover, in the comparison with the general population,* the presumed ten-year risk is remarkably increased in hemophiliacs (6.7 % vs. 8.9 %), indicating more unfavourable cardiovascular complication risk profile in patients with hemophilia.
Aims: To report the *case of* the patient with hemophilia presenting with thrombotic event.
Methods: The 60-year-old patient with moderate hemophilia A, smoker with arterial hypertension, increased serum level of cholesterol and inherited thrombophilia, immobilized because of hemophilic arthropathy, treated with on demand FVIII concentrate complained of the dull pain in epi- and mesogastrium irradiating to the back without any nausea, vomitus and fever is presented.
Results: The computed tomography scan revealed an unusual finding - an irregular thrombus almost in whole segment of the abdominal aorta. Combination of a*ntithrombotic drugs* and lifestyle changes led to disappearance of the his symptoms. During the follow up, he has not developed another thrombotic episode.
Conclusions: The life expectancy of hemophiliacs is closer to that of the general population. However, evidence-based guidelines are missing. Therefore, the coincidence of this disorder and thrombosis ought to be studied predominantly in older hemophiliacs with more cardiovascular risk factors. The management of cardiovascular events in hemophiliacs is the balancing game especially challenging in the situations when antithrombotic treatment or surgery is planned. Thus, prominent question is how to optimalize simultaneous antithrombotic and substitution therapy in patients with such coexistence of opposing hemostatic disorders.
We thank the support of project Vega 1/0168/16

FVIII54

Managing hemophilia treatment in algeria, the experience of constantine

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Background: - In Algeria there are 1943 people with hemophilia.
- The hemophilia and bleeding disorder center of Constantine was created in January 2009.
Aims: - Provide health education to patients and their families.
- Standardize the management of hemophilia.
- Prevent the occurrence of a hemorrhagic syndrome during a medical or surgical procedure.
- Ensure adequate management of bleeding.
- Prevention of hemophilic arthropathy.
Methods: Patients, the number of people with hemophilia followed in our center until 30/10/14 is 201 patients; 158 patients with hemophilia A and 43 with hemophilia B
- The guideline of treatment in our center:: A/Prophylaxis treatment: Inspired by the Canadian protocol and the 2006 recommendations from COMETH, It's used for children and adolescents; the tertiary prophylaxis is used in young adults.
B/On-demand treatment: patients which are not in prophylaxis.
Results: This evaluation was carried out in October 2014.
1/Hemophilia A: 26 patients receive prophylactic therapy (4 are in primary prophylaxis and 22 in secondary prophylaxis)
- Orthopedic clinical score PedNet: Initial 110/550 and the actual 61/550).
- Pettersson radiological score: initially 00/26 and remains the same.
Tertiary prophylaxis for adults: 5 patients, the number of bleeding episodes was significantly decreased and quality of life was improved.
2/Hemophilia B: 5 children, PedNet clinical joint score: initially 18/125 and the actual 10/125.
3/Complications: 11 patients on demand treatments have ACC and receive bypassing factors.
Conclusions:

- Prophylaxis reduced the incidence and severity of joint bleeds.
- It preserves joint function and improves quality of life.
- It's an expensive treatment but is profitable in the long term (eliminates the high cost of subsequent management of patients with joint damage).
- May prevent people with hemophilia experiencing severe disabilities.

FVIII55

Epidemiological Study of hemophilia at tlemcen university hospital (Algeria)

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Background: Hemophilia is a bleeding disorders X-linked caused by a deficiency of factor VIII (hemophilia A) or factor IX (hemophilia B). It causes hemorrhagic manifestations affecting the musculoskeletal system essentially in the severe form making the patient a physical disabled in absence of treatment.
Aims: Study the epidemiology, clinical manifestations depending on the degree of severity of hemophilia, analyze the complications of the disease and of its treatment and evaluate the therapeutic management.
Methods: This is a retrospective descriptive study of patients with hemophilia followed in our hematology department from May 1989 to December 2015.Statistical analysis was performed using the SPSS statistics software 20. The graphs were plotted by the Microsoft Office Excel 2013.
Results: This study concerns 90 hemophiliacs, Hemophilia A 71 cases, B19 cases.

Table Distribution according hemophilia type and its deg.

Hemophilia type	Severe form	Moderate form	Minor form	Total
HEMOPHILIA A	52	16	3	71 (79%)
HEMOPHILIA B	10	8	1	19 (21%)
TLEMCEN TOTAL	62 (69%)	24 (26%)	4 (5%)	90 (100%)
ALGERIA TOTAL	1161 (63%)	406 (22%)	276 (15%)	1843 (100%)

Median age is 25 years with extremes between (03 months and 67 years). 72% hemophiliacs were diagnosed before the age of 2 years in the severe form. The circumcision revealed hemophilia in 1/5 cases. We found 19 sporadic cases. The hemarthroses are the apanage of severe form (81%). The hemophilic arthropathy was found in 27 patients. 9 synoviorthesis were performed, but no surgery. The serology revealed 12% of HCV +,2% of HIV +, and 0% of HBS+ . The search of circulating antibody was positive in 15% of cases. We recorded 8 deaths. For the therapeutic we used fresh frozen plasma and cryoprecipitate in the1990, then hemophiliacs were treated by plasmatic factors received on demand. The prophylactic treatment was started in 2008.Twenty children are under secondary prophylaxis and 7 smaller ones are under primary prophylaxis with recombinant factors.
Conclusions: Hemophilia remains a rare disease but serious in its consequences on the functional and vital plan The application of the prophylactic treatment essentially in severe form for very young hemophiliacs allows us to hope for a better future for them pending to heal 1 day hemophilia by gene therapy.

FVIII56

Potency estimates for recombinant Factor IX in the one-stage clotting assay are influenced by more than just choice of APTT reagent

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Background: Potency discrepancies with one stage clotting assays (OSCA) for new recombinant and modified factor IX (FIX) therapeutics have been ascribed to the choice of activated partial thromboplastin time (APTT) reagent. Results from the collaborative study for the replacement of the 4th International Standard (IS) for FIX Concentrate showed that in two laboratories using the same APTT reagent (Dapttin), up to 20% difference in potencies for the recombinant FIX (rFIX) was found. However, good agreement of potency estimates were obtained for the plasma-derived products (pdFIX).
Aims: The present study was designed to identify reasons behind these discrepancies.
Methods: The two rFIX candidates from the collaborative study, together with two pdFIX preparations, were included and potencies were assessed using OSCA with 3 different APTT reagents relative to the 4th IS for FIX. Results were analysed by parallel line assay.
Results: Overall, SynthAFax yielded lower potencies for rFIX than SynthASil and Dapptin. A study of activation times showed that for Dapttin, the rFIX potency estimates decreased with increasing activation time (see Table 1) with a 10% and 20% reduction for 240 s and 300 s, respectively, compared to 120 s. A small reduction in rFIX potency over time was observed with SynthASil, however only rFIX2 showed the same effect with SynthAFax.
Further investigation using Dapptin showed that pre-diluent (haemophilic plasma or buffer) did not influence the potencies of rFIX, however, up to 10% lower estimates were obtained for rFIX when using a different source of the substrate haemophilic plasma.

Table 1 (Abstract FVIII56)

	Potency in IU/ml (%GCV)								
	Dapttin			SynthASil			SynthAFax		
Sample	120 s	240 s	300 s	120 s	240 s	300 s	120 s	240 s	300 s
rFIX1	11.51 (0.83%)	10.16 (4.23%)	8.93 (2.28%)	11.29 (4.57%)	10.96 (7.70%)	10.67 (2.31%)	7.50 (2.96%)	7.20 (2.35%)	7.12 (3.96%)
rFIX2	11.27 (8.36%)	9.53 (3.68%)	8.44 (7.28%)	10.89 (4.50%)	10.48 (6.62%)	9.88 (4.55%)	7.23 (4.36%)	6.22 (6.25%)	6.46 (1.38%)
pdFIX1	10.25 (5.66%)	10.14 (2.15%)	10.33 (7.06%)	10.83 (1.05%)	10.74 (5.79%)	10.64 (4.39%)	10.56 (2.43%)	10.27 (3.13%)	10.11 (2.50%)
pdFIX2	12.49 (3.09%)	12.73 (3.15%)	12.36 (5.48%)	13.08 (2.00%)	13.21 (4.90%)	13.08 (2.52%)	13.06 (1.20%)	13.11 (1.60%)	13.10 (4.14%)

Conclusions: This study highlights that different potency estimates can be obtained, even when using the same standard and APTT reagent, and that careful validation of assay methods is required.

FVIII57

Calcium chloride reagent regulates thrombin generation response of hemophilia plasma to factor concentrate treatments

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Background: Fluorogenic thrombin generation test (TGT) is a global hemostasis assay with a potential to predict bleeding tendencies and treatment effect in patients with blood coagulation disorders. Despite 15 years of clinical research, the value of these diagnostic applications remains controversial, possibly due to suboptimal sensitivity to coagulation deficiencies, robustness and reproducibility.
Aims: The goal of this study was to investigate the effect of calcium chloride ($CaCl_2$) reagent concentration on the TGT's response to concentrates of coagulation factors (F) VIII, IX, XIa and VIIa.
Methods: Plasma from coagulation factor deficient patients was supplemented with coagulation factors and tested in a commercial calibrated automated TGT and in-house versions of TGT under different $CaCl_2$ conditions.
Results: Thrombin peak height (TPH) was strongly dependent on the concentration of $CaCl_2$ in a commercial as well as in-house TGT assays. In our experiments, a commonly used $CaCl_2$ concentration of 16.7 mM was positioned on a declining portion of the bell-shaped $CaCl_2$ dose-response curve. For example, a 20% decrease in $CaCl_2$ concentration in a FIX-supplemented FIX deficient plasma was associated with a 30% decrease in TPH. However, the concentration of $CaCl_2$ needed to maximize the TPH did not coincide with the concentration needed to maximize the ratio of the TPH in factor supplemented vs. TPH in factor deficient plasma. The range of 12–14 mM of $CaCl_2$ was optimal to measure TGT responses of hemophilia plasma to factor concentrate treatments.
Conclusions: Variations in $CaCl_2$ concentration in the assay mixture (and sodium citrate concentrations in patient plasma samples) may affect TGT responses and sensitivity to factor deficiencies, and lead to increased inter- and intra-laboratory variance. Therefore, implementation of TGT by clinical and quality control laboratories my require optimization and control of $CaCl_2$ concentration.

FVIII58

Inherited bleeding disorders: an experience from a tertiary care centre in south india with emphasis on an algorithmic approach to diagnosis

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Background: Inherited bleeding disorders are a heterogenous group comprising disorders of primary and secondary hemostasis. These have varied presentation and require a step-wise approach starting with screening tests and moving towards specific confirmatory tests. A near-normal Prothrombin Time (PT), activated Partial Thromboplastin Time (aPTT) and Platelet count (PC) with or without prolonged Bleeding Time (BT) points towards primary hemostatic defect while a prolonged PT and/or aPTT points towards a secondary hemostatic defect.
Aims: To analyse the spectrum of inherited bleeding disorders with respect to their prevalence and clinical profile and elucidate a step-wise algorithmic approach for diagnosis in resource limited set ups.
Methods: This was a preliminary record-based descriptive study over a period of 6 years (Mid 2009–2015) from a single large tertiary care centre in South India. Only inherited bleeding disorders were included. The clinical details and coagulation work-up were retrieved from records. The diagnoses were based on coagulation screen, mixing studies, factor assays, clot retraction test and platelet aggregation studies in varying combinations. This served as a gap analysis to add new tests and an attempt was made to prepare an algorithm for systematic work up of these disorders.

Table 1 Prevalence and clinical profile of inheri

Bleeding disorder- n (%)	Type of defect	Clinical Presentation
von Willebrand disease- 10(6.8%) Glanzmann thrombasthenia-7 (4.7%) Bernard-Soulier syndrome-2(1.4%)	Primary hemostatic	Epistaxis (89%) Prolonged bleeding after trauma (73%) Gum bleeding (47%) Recurrent ecchymosis (36%)
Hemophilia A-103(69.6%) Hemophilia B -12(8%) Factor XI deficiency-1(0.7%) Combined Factors VIII/IX deficiency-1(0.7%)	Intrinsic pathway	Hemarthrosis (49%) Prolonged bleeding following surgery (28%) Dental bleeding (9%) Hemetemesis (4%), Melena (2%) Post circumcision bleed (2%)
Factor VII deficiency-1(0.7%)	Extrinsic pathway	Epistaxis, Prolonged bleeding following trauma (100%)
Factor X deficiency-1(0.7%)	Common pathway	Intracranial bleed (100%)
Factor V deficiency -4(2.7%)	Common pathway	Hemarthrosis (50%) Post partum hemi peritoneum, PV bleed (25%) Bleeding Inguinal and groin ulcers and recurrent epistaxis (25%)
Prothrombin deficiency -2(1.4%)	Common pathway	Recurrent epistaxis, ecchymosis, gum bleeding, prolonged bleeding after trauma (100%) Menorrhagia (50%)
Fibrinogen deficiency - 3(2%)	Common pathway	Prolonged bleeding following trauma (100%) Epistaxis (33%) Intracranial bleed (33%)
Factor XIII deficiency- 1(0.7%)	-	Epistaxis, bruising after trauma, history of umbilical stump bleeding in day 3 of life, Melena (100%)

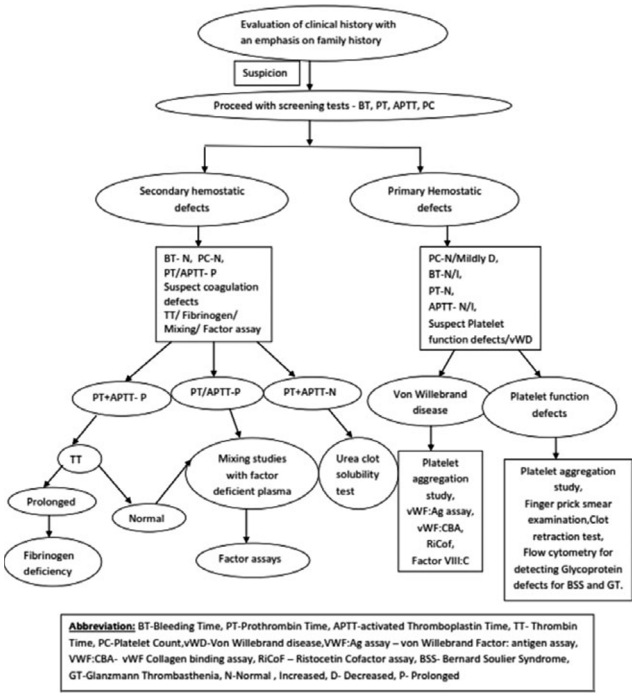

Figure 1 Diagnostic algorithm for inherited bleedi.

Results: There were 148 patients in total with mean age at diagnosis being 14.4 years (SD 13.98) with 87.8% males and 12.2% females. Positive family history was obtained in 42% cases. Primary and secondary hemostatic defects accounted for 19 (12.8%) and 129 (87.2%) cases respectively with overwhelming majority being Hemophilia A (69.6%) cases. The distribution of cases and patterns of bleeding is summarised in Table 1. The proposed diagnostic algorithm is depicted in Figure 1.
Conclusions: A varied spectrum of bleeding disorders were encountered with Hemophilia A being the commonest. The diagnosis of these disorders can be arrived at by a systematic algorithmic approach.

FVIII59

A new comprehensive screening program for optimizing the diagnosis of coagulopathies in a tertiary hospital

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Background: With more than 1100 beds and a reference area of 1.200.000 inhabitants, our hospital is one of the biggest tertiary centers in our country and the haemostasis laboratory is processing more than 1000 samples per day.
Aims: Given this enormous workload, optimizing the diagnosis efficiency of coagulopathies is crucial.
Methods: We designed a computational algorithm through implementation of a new laboratory high throughput technology. This algorithm includes the generation of an electronic alert (*FIN: factors if necessary)* when a prothrombin time *(PT)* or activated partial thromboplastin time *(APTT)* alteration not known or justified are detected. Subsequently, the remaining plasma samples are frozen until the haematologist reviews the patient's clinical data and generates the appropriate test: lupus anticoagulant *(LA)*, intrinsic or extrinsic coagulation factor pathway *(IFP, EFP)*. Preliminary analysis included all consecutive cases with *FIN* from July 2014 to August 2015.
Results: 1435 cases with FIN were included in the study, among them 1183 patients showed APTT ratio > 1.3 and 515 showed PT ratio > 1.2. After the haematologist assessment, 418 samples were processed for *IFP*, 407 for *LA* and 110 for *EFP*. 319 patients (22%) were finally analysed for factor deficiency characterization. While in the *IFP*, only a unique or double deficiency was considered for review (FVIII: 50, FIX 19, FXI: 42, FXII: 53), most of the cases with PT alteration had multiple factor defects. A total of 140 patients had a *LA* positive determination. We could detect, even molecularly characterized in some cases, 59 coagulation defects (21 LA, 7 mild haemophilia A, 5 Von Willebrand diseases, 4 FXI, 18 FXII and 4 FVII deficiencies).
Conclusions: Our study provides a new comprehensive screening algorithm that markedly improves the diagnosis efficiency of coagulopathies in a tertiary hospital and contributes to importantly diminish redundant analytical determinations.

FVIII61

Distraction arthroplasty in patient with hemophilic arthropathy (a case report)

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Background: Ankle *distraction arthroplasty* is an innovative procedure for treating ankle arthritis in selected patients. *Distraction arthroplasty* stretches the joint a part for a period of time.
Aims: This technique is used to unload the ankle joint and allow healing of the damaged joint. This method is promising treatment approach for ankle osteoarthritis. Literature data are limited.
Methods: Case 1: family history: mother's father - prolonged bleeding after tooth extraction in elder age, no laboratory test, sister - carrier, two sons - hemophilia A. Personal history: From birth increased formation of hematomas, epistaxis, after exercise pain, swelling of the ankles after playing football, spontaneous joint bleeding. Replacement therapy: rFVIII (moroctocog alfa) Age 29 years: orthopedist evaluated hemophilic arthropathy, arthrosis of ankle joints IV degree. Strategy: Distraction arthroplasty -The external ring fixator extends articular gap,- Joint mechanism allows flexion and extension of the ankle joint during fixation,- 12 weeks, - The expected effect - generation of new cartilage, elimination of pain, Hematology - treatment:

1) Replacement therapy rFVIII, Ensure the hemostasis during surgery and rehabilitation,
2) Maintain FVIII 80% 2. - 7. postoperative day,
3) Maintain FVIII 30% 12 weeks after surgery,
4) After 12 weeks - administration of autologous platelet-rich plasma.

Results:
1 Ensure the hemostasis during surgery and 24h after the surgery, Mean daily dose rFVIII 133,2 IU/kg a 6h,
2 Maintain FVIII 80% 2. - 7. day after surgery. Mean daily dose 57,35 IU/kg a 6–8h,
3 Maintain FVIII 30% 12 weeks, Mean daily dose 16,66 IU/kg a 8–12h. No bleeding complications.

Conclusions: Ankle *distraction arthroplasty* is an innovative procedure for treating ankle arthritis in selected patients with hemophilic arthropathy and secondary osteoarthrosis. rFVIII provides fast and effective treatment. Surgery and rehabilitation were successful without bleeding complications.

Factor XI and the Contact System

FXI01

Novel mechanisms regulating Factor XI plasma levels

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Background: Coagulation factor XI (FXI) has become increasingly interesting for its role in pathogenesis of thrombosis. While elevated plasma levels of FXI have been associated with venous thromboembolism (VTE) and ischemic stroke (IS), its deficiency is associated with mild bleeding.
Aims: To investigate novel genetic and post-transcriptional regulatory mechanisms of FXI plasma level that could serve as therapeutic targets for thrombotic disease treatment and prevention.
Methods: We performed genome-wide association study (GWAS) for plasma FXI levels by increasing sample size 40-fold from previous studies, and we have used novel data imputed to the 1000 Genomes (1000G) reference panel. Individual GWAS analyses adjusted for age, sex and population stratification, including a total number of 16,169 European individuals from ARIC, GHS, MARTHA and PROCARDIS studies, were meta-analyzed and further replicated in 2,045 individuals from the F5L family, GAIT2 and MEGA studies, and additional association with activated partial thromboplastin time (aPTT) was tested for the top SNPs. In addition, a study on the effect of microRNA (miRNA) on FXI regulation was performed using *in silico* prediction tools and *in vitro* luciferase assays in a hepatic cell line.
Results: Three loci showed robust association to circulating FXI levels: *KNG1* (rs710446, $P = 2.86 \times 10^{-295}$), *F11* (rs4253417, $P = 7.31 \times 10^{-188}$), and *GCKR* (rs780094, $P = 7.12 \times 10^{-9}$), the first two also associated with aPTT. Additionally, eight miRNAs were predicted to bind F11 mRNA. Over-expression of either miR-145 or miR-181 significantly reduced the luciferase activity in cells transfected with pLS-FXI-3'UTR.
Conclusions: Our results confirm *KNG1* and *F11,* and discover *GCKR,* which has been reported to have pleiotropic effects in other coagulation proteins, as novel interesting loci regulating plasma FXI levels. Finally, we also demonstrate post-transcriptional regulation of FXI protein expression by miRNAs.

FXI02

Plasma kallikrein contributes to tissue plasminogen activator (tPA) therapy-induced brain injury during thrombotic stroke

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Background: Although prompt thrombolysis with tPA can improve clinical outcomes following ischemic stroke, the benefits of this therapy are often limited by tPA's effects on neuronal injury and intracerebral hemorrhage. Plasmin has been reported to activate the contact system, which may contribute to the effects of tPA during thrombolysis. However, while plasma kallikrein (PKal) has been implicated in both ischemic and hemorrhagic stroke, the role of PKal in brain injury associated with tPA therapy is not yet available.
Aims: We investigated the effects of tPA on PKal activation and the role of PKal on cerebral outcomes in a murine thrombotic stroke model treated with tPA.
Methods: PKal activation *in vivo* was analyzed in wild type (WT) and PKal-deficient ($Klkb1^{-/-}$) mice subjected to intravenous infusion of tPA and *in vitro* in normal and plasmin-, PKal-, and FXII-depleted human plasma. Stroke was induced by photothrombotic laser in WT and PKal-deficient ($Klkb1^{-/-}$) mice. Mice were administered with tPA in the absence or presence of co-administration with a selective PKal inhibitor. Mice were sacrificed at 24 h post-stroke to evaluate brain injury.
Results: Administration of tPA increased PKal activity in WT mice and this response was not observed in $Klkb1^{-/-}$ control mice. PKal activation by tPA in normal human plasma was reduced by 72 and 40% in FXII- and plasmin-deficient plasma, respectively. Acute tPA administration at 2 h post stroke increased infarct volume, edema and hemorrhage transformation when compared with the untreated stroke group in WT mice ($P < 0.05$) and compared to $Klkb1^{-/-}$ mice with stroke receiving tPA ($P < 0.05$). In WT mice with stroke, pretreatment with a PKal inhibitor prior to tPA administration decreased tPA-induced infarct volume, edema and hemorrhage in a dose dependent manner ($P < 0.05$).
Conclusions: tPA increases PKal activity via a plasmin- and FXII-dependent mechanism. Co-administration of a PKal inhibitor with tPA may reduce brain injury during thrombolytic therapy for stroke.

FXI03

Role of plasma kallikrein-kinin system in the pathogenesis of ambient particulate matter-induced lung injury

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Background: Plasma kallikrein-kinin system (KKS), consisting of high-molecular-weight-kininogen (HK), prekallikrein (pKal) and Factor XII (FXII), is important in many pathophysiological processes, including inflammation, coagulation and tissue injury. Particulate matter (PM) is a key component of air pollutants and is associated with mortality of cardiovascular and respiratory diseases. PM-induced tissue injury involves inflammation and coagulation. A recent *in vitro* study has shown that PM induced FXII activation and thrombin generation.
Aims: This study was aimed at determining the role of plasma KKS in the pathogenesis of PM-induced lung injury using genetically-modified mouse models.

Methods: Plasma was incubated with $PM_{2.5}$, followed by detection of HK cleavage by Western blotting, measurement of bradykinin (BK) by ELISA, and evaluation of kallikrein (Kal) activity using chromogenic substrate S-2302. Thrombin generation was measured by calibrated automated thrombogram. Intratracheal instillation of $PM_{2.5}$ was used to induce lung injury. Cytokines in bronchoalveolar lavage fluid (BALF) was measured by ELISA. The paraffin sections of lung were evaluated using H&E staining.
Results: In a dose-dependent manner, $PM_{2.5}$ significantly induced HK cleavage, BK elevation, and Kal activation in human and mouse plasma. $PM_{2.5}$-induced HK cleavage in plasma was completely blocked by Kal and FXIIa inhibitors, as well as in FXII- and pKal-deficient plasma. $PM_{2.5}$ markedly induced thrombin generation in wild-type mouse plasma. Both inhibition and deficiency of FXII and pKal completely blocked $PM_{2.5}$-induced thrombin generation. In $PM_{2.5}$-induced lung injury model, $pKal^{-/-}$ mice exhibited a decrease in both IL-6 level in BALF and histologic lung injury score. In contrast, $FXII^{-/-}$ mice did not show any amelioration of lung injury.
Conclusions: $PM_{2.5}$ activates pKal and FXII to induce BK release and thrombin generation, however, pKal, but not FXII, contributes to $PM_{2.5}$-induced lung injury.

FXI04

Assessment of small molecular weight inhibitors of the contact pathway

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Background: There is growing evidence that the inhibition of the contact pathway, and FXIIa in particular, is of considerable interest in the safe prevention of medical device-induced thrombosis as well as in TF-driven blood coagulation assays. Among FXIIa inhibitors, peptides, antibodies or antisense nucleotides are widely known. In contrast, there are only few reports describing small molecular weight inhibitors.
Aims: We recently described a series of coumarins targeting FXIIa. In this study, we aim to investigate the anticoagulant and antithrombotic properties of two soluble derivatives.
Methods: The IC_{50} of the compounds were first determined on various coagulation proteases. Then, their behavior in plasma was studied (at 500 μM) with aPTT and PT tests and with the thrombin generation assay (cTGA). They were assessed in whole blood (at 100 μM) using a microfluidics model dedicated to the study of the contact pathway. Finally, one compound was tested in a rat model of venous thrombosis (at 10 mg/kg).
Results: The compounds were 10–25-fold more active on the contact phase proteases (6-13 μM) than on thrombin or FXa (150–230 μM). In plasma, the molecules had no effect on the PT while the aPTT was nearly doubled. In the cTGA, they showed a greater anticoagulant effect on the contact phase than on the TF-pathway. Then, the compounds were assessed in blood with microfluidics. When blood was perfused on a kaolin/collagen surface, they delayed and decreased the fibrin formation without modifying the platelet function. Finally, the molecule tested in rats showed a relevant antithrombotic effect. Taken together, these results suggest that the compounds are organic small inhibitors of the contact pathway.
Conclusions: The compounds are interesting small inhibitors of the contact phase, but their potency and selectivity must be improved. Based on our model of FXIIa, we are currently developing compounds with potentially increased selectivity towards FXIIa.
Funding: FRS-FNRS Belgium

FXI05

Copurification of polyphosphate with nucleic acids may contribute to the procoagulant activity of DNA and RNA

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Background: Polyphosphate (polyP) is widespread in prokaryotic and eukaryotic cells. Both polyP and extracellular nucleic acids are implicated in modulating coagulation, although their relative contributions are not definitively determined. Isolating nucleic acids from cells usually exploits the anionic properties of these molecules, which are shared by polyP.
Aims: 1) Determine whether polyP co-purifies with DNA or RNA using commercial kits to isolate nucleic acids from cellular or tissue sources.
2) Determine if polyP contributes to the in vitro procoagulant activity of nucleic acids.
Methods: DNA and RNA were purified from mammalian cells using commercial kits. Samples were treated with nucleases or exopolyphosphatases to determine the relative contributions of nucleic acids and polyP. The procoagulant activity of these nucleic acids was compared to that of synthetic nucleic acids with and without added polyP. *In vitro* clotting assays compared the abilities of various preparations to activate the contact pathway and to abrogate the anticoagulant activity of tissue factor pathway inhibitor.
Results: Long-chain polyP was markedly more potent on both a mass and molar basis than nucleic acids as a contact activator. Genomic DNA was a stronger contact pathway activator than cellular RNA. Pre-treatment of some nucleic acid preparations with exopolyphosphatase significantly reduced their procoagulant clotting activity, but pre-treatment with nucleases also reduced their clotting activity.
Conclusions: PolyP present in cells or tissues is likely to co-purify with nucleic acids using commercially-available nucleic acid purification kits. This contaminating polyP can contribute to the observed procoagulant activity of nucleic acids. Experiments evaluating the procoagulant activity of RNA or DNA isolated from cellular or tissue sources should include controls to eliminate the effects of possible polyP contamination.

FXI06

Correlation between the thrombogenic Factor XIa and the thrombus score in the venous stasis rabbit model

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Background: Activated factor XI (FXIa) has been identified as the probable causative agent associated with thromboembolic events (TEE) following intravenous (IV) IgG (IVIG) product administration. Although there is no official method required by the regulator to validate the therapeutic safety of new batches of IVIG, the rabbit venous stasis assay, a prothrombogenic model, remains the model of choice for *in vivo* assessment of potential TEE.
Aims: This study aimed at standardizing the in vivo venous stasis rabbit model and quantifying the formed thrombi on a ranking system to evaluate the amount of FXIa in new batches of IVIG, in order to predict potential TEE.
Methods: Anesthetized rabbits (2.5 to 3 kg) were infused with 10 ml/kg of 200 mM glycine, 5% BSA (Gycine-BSA) solution spiked with increasing doses of FXIa followed by a jugular venous stasis of 2-cm long for 15-min. The formed thrombus was scored according to a ranking system from 0 to 4.
Results: Spiked Glycine-BSA solution with 4 increasing doses of FXIa (0, 1, 1.5, 2 and 7 mU/mL) showed a dose-dependent clot formation response of 0, 0.5, 1.0, 2.33, and 3.75 respectively. The IVIG Gamunex and spiked Gamunex with 1.5 mU/mL of FXIa scored 0.8 and 3.5

respectively. Concomitantly, the bleeding time in rabbits treated with the spiked Glycine-BSA decreased from 220 s to 130 s with increasing doses of FXIa.
Conclusions: The current study demonstrates that the standardized venous stasis rabbit model is very sensitive to FXIa and that the thrombus score correlates with the amount of FXIa present in the test solution. Furthermore, this standardized model can be used to predict the potential for a TEE in batches of IVIG.

FXI07
Procoagulant activity in immunoglobulin products

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Background: Thrombotic events have been associated with the administration of Immunoglobulins (Igs) and the procoagulant activity (PA) was attributed to FXIa, a process related impurity.
Aims: We used a number of assay methods including FXIa chromogenic assay (FXIaCA), Non Activated Partial Thromboplastin Time (NAPTT) and thrombin generation assay (TGA) to detect PA in Igs.
Methods: 20 batches of clinical Igs from 4 manufacturers were tested for PA. Two commercial FXIaCA with different sensitivity to FXIa (LoQ- Rossix kit: 0.3 mIU/ml; Hyphen kit: 5 mIU/ml) are available. TGA can be as sensitive as the Rossix FXIaCA, but difficult to obtain quantitative results and agreement between laboratories. NAPTT in FXI deficient plasma or pooled normal plasma (PNP) are much less sensitive and also difficult to standardise and perform quantitatively. PA in Igs can be quantified in FXIaCA and NAPTT relative to the International Standard for FXIa and expressed in mIU FXIa. However, PA detected by TGA can only be expressed in readouts such as lag time or peak thrombin.
Results: Variable amounts of FXIa were detected in the Igs by the Hyphen FXIaCA ranging from < LoD to 35 mIU FXIa/ml, similar trends were obtained by NAPTT using FXI deficient plasma (< LoD to 38 mIU FXIa/ml) and TGA using FXI deficient (< LoD to 272.5 nM peak thrombin) and PNP (105–231 nM peak thrombin, buffer blank = 118 nM peak thrombin). However, the batches of Igs that showed undetectable activity in FXIaCA, NAPTT using FXI deficient plasma and TGA showed substantial shortening of clotting times, giving test to blank clotting time ratios of 0.8 in NAPTT using PNP.
Conclusions: In conclusion, batches of clinically available immunoglobulin product still contain procoagulant activity which could be attributed to FXIa. However, the shortening of the NAPTT in PNP by some of these batches show that procoagulant components other than FXIa could be present in these products.

FXI08
Investigation of possible correlation between clinical and laboratory phenotype in congenital FXI deficiency: results from a single center

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Background: Bleeding phenotype (BP) in FXI deficient patients (pts) is not correlated with FXI:C level. There is no optimal predictive laboratory test to assess bleeding risk in such pts.
Aims: To analyze whether platelet function testing and global coagulation assays can serve as clinical tools in predicting BP in FXI deficient pts.
Methods: The ISTH-Bleeding Assessment Tool (BAT) was used to classify bleeders (score >3 for males, >5 for females) and no-bleeders. To explore platelet function, PFA-200 System and Light Transmission Aggregometry (LTA) were utilized. Thromboelastography (TEG) and Calibrated Automated Thrombography (CAT) were performed. The association between continuous variables and BP was tested by means of logistic regression model. Pts were evaluated also for molecular biology.
Results: We enrolled 25 pts: 7 bleeders, 18 no-bleeders. Bleeders median FXI:C: 10.1% (4.3–46%); no-bleeders median FXI:C: 27% (6.2–67%). No significant association was found between FXI:C levels and BP (OR 0.968; 95% CI 0.92–1.01; p = 0.19). Regarding PFA-200, a trend was observed, for association between BP and Collagen/Epinephrine cartridge test results (p 0.052); concerning LTA, a trend was observed, for association between BP and ADP 5 μM (p 0.07) and Adrenaline 5 mM (p 0.07) test results. Concerning CAT parameters no significant associations were found with BP: endogenous thrombin potential, p 0.43; lag time, p 0.37; peak, p 0.28; time-to-peak, p 0.42. Regarding TEG parameters, no significant associations were found with BP: R, p 0.58; a-Angle, p 0.26; MA, p 0.38; Ly30, p 0.85; Ly60, p 0.36. Eighteen pts were studied for molecular biology: 7 had homozygous mutations, 3 compound heterozygous mutations, 6 heterozygous mutations. In 2 pts mutations were not detected.
Conclusions: We confirm variability in BP among FXI deficient pts, not significantly related to FXI:C levels. We found a trend for platelet dysfunction (PFA200 and LTA) and BP. We did not find any significant association between BP and TEG or CAT parameters.

Factor XIII and Fibrinogen

FXIII02
Structure and function consequences of a novel missense mutation in *FGB* gene (Bbeta Gly272Arg/p.Gly302Arg) Resulting in Afibrinogenemia

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Background: Inherited fibrinogen disorders are rare and result in quantitative (afibrinogenemia, hypofibrinogenemia) or qualitative (dysfibrinogenemia) fibrinogen deficiency or both (hypodysfibrinogenemia).
Aims: Characterization of a novel missense mutation in fibrinogen betta chain.
Methods: Here we present a case report of a 25 years old Caucasian patient from Argentina with a history of bleeding since childhood. Fibrinogen levels were undetectable when evaluated with both, a functional as well as an antigenic method. Fibrinogen genes have been analyzed by direct sequencing. A mutant protein was analyzed using fibrinogen crystal structure (PDB ID: 3ghg).
Results: Direct sequencing of fibrinogen genes demonstrated a novel homozygous point mutation in the *FGB* gene (Gly272Arg, HGVS nomenclature: p.Gly302Arg). An examination of the local environment and neighboring sequence of the wild type residue (Gly272) in the fibrinogen crystal structure (PDB ID: 3ghg) showed that the mutation occurs in a highly conserved region which preserves the core fold of the C-terminal beta chain. The C-terminal part is important for the proper secretion of the beta chain. A mutation especially to a large positively charged Arg residue in this area would most likely disturb the core fold by either of the two mechanisms: i) when accommodated inside the core fold the large positive side chain will form additional interactions with adjacent residues (Asp261, Asp267, Asp281) thereby

disturbing the local structure ii) if the residue lies on the surface its positive charge may promote non-native interactions with other proteins hindering the action of molecular chaperons important for secretion. Either ways the regular secretion of the beta chain would be disturbed.
Conclusions: A novel missense mutation (Bbeta Gly272Arg) was identified in a highly conserved region of the beta chain that results in afibrinogenemia most probably due to its effect on the secretory process of the fibrinogen beta chain.

FXIII03
Visual quantification of Fibrin network density

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Background: Fibrin network structure is an important determinant of thrombotic and bleeding risk. Scanning electron microscopy (SEM) images of clots provide useful information about fibrin network structure. However, quantification of these images is complicated: automated software is often not reliable, and manual quantification is too laborious to get sufficient numbers for clinical studies.
Aims: To develop a reliable and fast method to quantify fibrin network density of clots analyzed by SEM.
Methods: All patients gave written informed consent and the study was approved by the local ethical committee. In series I, whole blood from 32 patients taking vitamin K antagonists (VKAs) was mixed with 1 pM tissue factor and 16.7 mM $CaCl_2$ (final concentrations) and allowed to clot for 50 min at 37°C. Clots were fixed and visualized by SEM. Fiber thickness was determined manually using ImageJ software. Fibrin network density was quantified using a 1-to-5 scoring system: a score of 1 was given to an open and a score of 5 to a tight fibrin structure. In series II, fibrin density will be studied in hemophilia patients and healthy controls. SEM images will be scored by six independent observers at three different centers. The fibrin density of clots from hemophilia patients and controls will be compared, and agreement between the observers will be evaluated to validate the method.
Results: In clots from patients taking VKAs (series I), fibrin fiber thickness correlated with fibrin network density (Spearman $r = -0.47$, $P = 0.0062$). The outcomes of the hemophilia study (series II) will be presented.
Conclusions: In clots from patients taking VKAs, manual measurements of the fiber thickness correlated with visual scoring of the fibrin clot density. Visual scoring is less time consuming than fiber thickness measurements. Our preliminary results show visual scoring could replace manual quantification of fibrin clots. The outcomes of the hemophilia study will provide further insight about the validity of the new scoring system.

FXIII04
Development and evaluation of total and free Factor XIII-B subunit ELISA methods

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Background: Coagulation factor XIII (FXIII) is a tetramer of two catalytic FXIII-A and two protective/inhibitory FXIII-B subunits (FXIII-A_2B_2). FXIII A and B subunits form 1:1 complex in the plasma; 99% of FXIII-A subunits are in complex, while FXIII-B is present both in complex and in free form. Complex formation with FXIII-B subunit significantly prolongs the half-life of FXIII-A_2 in the circulation and prevents its spontaneous activation.
Aims: To develop and evaluate ELISA methods developed for the determination of total and free FXIII-B subunits in human plasma.
Methods: Three monoclonal antibodies were developed and used to design two sandwich type methods. Two antibodies were biotin-labeled (anti-free FXIII-B and anti-total FXIII-B) and used to capture FXIII-B from the samples. The third, HRPO-labeled antibody, which recognizes both FXIII-B forms equally well was used as tag antibody in both assays. Assays were performed in streptavidin coated microplates.
Results: The limit of quantitation was 0.66 ng/mL, the measuring range was 0.66–40 ng/mL for both assays using 1:1000 plasma dilution. Both assays showed good reproducibility (Table 1).

Table 1

	Total FXIII-B ELISA		Free FXIII-B ELISA	
	Within run CV% ($N = 20$)	Within laboratory CV% ($N = 20$)	Within run CV% ($N = 20$)	Within laboratory CV% ($N = 20$)
Control Norm.	2.16 (23.71 mg/L)	5.8 (23.71 mg/L)	2.10 (13.12 mg/L)	3.57 (13.12 mg/L)
Control Pathol.	2.99 (8.12 mg/L)	6.5 (8.12 mg/L)	3.01 (4.50 mg/L)	5.03 (4.50 mg/L)

The recovery of free FXIII-B and total FXIII-B assays were 93–140% and 93–103% respectively. In normal plasma samples ($N = 10$) measured (21.2 ± 4.3 mg/L) and calculated (20.9 ± 3.1 mg/L) total FXIII-B levels based on free FXIII-B (10.8 ± 2.0 mg/L) and FXIII-A_2B_2 (20.7 ± 3.2 mg/L) levels well agreed.
Conclusions: The methods are suitable for the measurement of total and free FXIII-B subunit antigen levels in the plasma both in normal and pathologic range with good precision.

FXIII05
FXIII activity and antigen during pregancy

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Background: There are limited data on the changes of factor XIII (FXIII) during pregnancy and comparability of different assay systems of FXIII.
Aims: Here, we compare the courses of FXIII activity (FXIII:Act) and antigen (FXIII:Ag) throughout pregnancy.
Methods: Samples were collected during first (T1, weeks 0–12), second (T2, weeks 13–28) and third trimester (T3, weeks 29–40) in 82 pregnancies in 67 patients (age: 31.2; 20–46). Pregnancies were divided into groups of patients with FXIII concentrate administration (FXIII-Adm) due to pregnancy related bleeding complications (n = 36) and without (n = 46).
Results: In patients without FXIII-Adm mean ± SD FXIII:Act was $106.9 \pm 17.1\%$ during T1, $104.0 \pm 14.1\%$ during T2 and $92.6 \pm 15.5\%$ during T3. Mean±SD FXIII:Ag was $102.5 \pm 14.5\%$ during T1, $91.0 \pm 12.1\%$ during T2 and $77.0 \pm 15.4\%$ during T3. In patients with FXIII-Adm mean±SD FXIII:Act was $104.6 \pm 13.9\%$ during T1, $99.8 \pm 13.6\%$ during T2 and $88.0 \pm 14.5\%$ during T3. Mean±SD FXIII:Ag was $97.7 \pm 13.9\%$ during T1, $88.0 \pm 11.5\%$ during T2 and $70.9 \pm 11.9\%$ during T3. There was no significant difference between FXIII:Act and FXIII:Ag in both groups and in all trimesters. In the group without FXIII-Adm there was a significant decrease of mean FXIII:Act from T1 and T2 compared to T3 ($P < .001$). Mean FXIII:Ag significantly decreased from T1 throughout T3 ($P < .001$). In the group with FXIII-Adm there was a significant decrease of mean FXIII:Act from T1 and T2 compared to T3 ($P < .001$) only, whereas mean FXIII:Ag decreased throughout all

trimesters ($P < .005$). In the group without FXIII-Adm correlation (R^2) of FXIII:Act and FXIII:Ag was .708 for T1, .605 for T2 and .793 for T3. In the group with FXIII-Adm R^2 was .480 for T1, $R^2 = .458$ for T2 and $R^2 = .774$ for T3.

Conclusions: FXIII:Act levels were higher than FXIII:Ag during whole pregnancy. During pregnancy discrepancy between Act and Ag increased from 5 IU/dL in T1 to 15 IU/dL in T3 regardless of FXIII-Adm. Thus, comparability of different assay systems is variable during pregnancy with or without FXIII-Adm.

FXIII06

Thromboelastography (TEG) and thrombin generation assay (TGA) in congenital Afibrinogenemia

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Background: Fibrinogen is a critical coagulation protein which is converted to fibrin to form the structure of a clot. Afibrinogenemia, a rare, autosomal recessive bleeding disorder, occurs when there is absence of measurable fibrinogen. Global hemostasis assays provide a detailed assessment of an individual's hemostatic state.

Aims: The aim of the study is to determine if a standard dose of 70 mg/kg of human fibrinogen concentrate (HFC) in afibrinogenemia patients is adequate to reach normal fibrinogen levels, and to evaluate thromboelastography (TEG) and thrombin generation assay (TGA) before and after infusion of HFC.

Methods: The study was IRB-approved and self-funded. Upon signing informed consent, subjects underwent a pharmacokinetic (PK) assessment following a dose of HFC 70 mg/kg. We assayed thrombin time, fibrinogen activity (Clauss) and antigen, kaolin-activated TEG and TGA with PPP reagent pre-dose and recovery. For subjects whose levels did not rise above 1.5 g/L, the HFC dose was adjusted and PK profile repeated.

Results: Of the 4 subjects (3 children and 1 adult), 2 (2 children) required an increase in HFC dose. TEG parameter MA demonstrated substantial improvement post-infusion. There was no change in TGA parameters. See tables for details.

Conclusions: The licensed dose of HFC is based on a small study. Our data suggest that individualized dosing based on fibrinogen levels may be necessary. The TEG parameter MA (the one sensitive for fibrinogen) improved in accordance with the correction of fibrinogen levels. In one subject whose fibrinogen recovery was insufficient (< 1.5 g/L) in visit 1, TEG parameters normalized suggesting that normalization of clot-forming ability can occur at subnormal fibrinogen levels and that TEG could be used to guide dosing in afibrinogenemia patients. TGA results were normal in all subjects, and were unaltered by HFC. This demonstrates, as expected, that patients with afibrinogenemia are able to generate thrombin normally.

FXIII07

Clinical and molecular characteristics of patients affected by congenital fibrinogen deficiency

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Background: Fibrinogen (FI) plays a crucial role in the haemostatic process for fibrin clot formation and platelet aggregation. It is a plasma glycoprotein consisting of two sets of three different polypeptide chains (Aα, Bβ, and γ, encoded by the FGA, FGB, and FGG genes, respectively). The mutational spectrum of these quantitative fibrinogen disorders includes large deletions, point mutations causing premature termination codons, and missense mutations often affecting fibrinogen assembly and/or secretion.

Aims: The study follows our previously published analysis of FI congenital deficient patients from Pakistan. Here we have identified the clinical and molecular characteristics of further fibrinogen deficient patients from our center.

Methods: Seven patients were analyzed for clinical and molecular characteristics. Plasma fibrinogen coagulant activity (Fg: C) was measured by clauss method.All axons and exon-intron junctions were PCR amplified from the genomic DNA of the probands and Sanger sequenced on both strands. Mutations were confirmed in the probands' relatives by sequencing the relevant regions.

Results: We analyzed 7 afibrinogenemic patients, all belonging to consanguineous families. In these patients, we identified: 3 nonsense mutations in FGA, one splicing and one nonsense mutation in FGG, and one missense mutations in FGB, shared by two affected siblings. All patients had grade III bleeding symptoms. (See Table).

Table Clinical and molecular characteristics of patients.

Proband	Gene/exon or intron	Genetic Variation	Native protein variation	Mature protein variation	Clinical features
P1	FGB/exon 7	g. 7075A>G	p. Tyr356Cys	p. Tyr326Cys	Haematoma, bruises
P1′s Sister	FGB/exon 7	g. 7075A>G	p. Tyr356Cys	p. Tyr326Cys	Bruises, umbilical cord bleeding
P2	FGG/exon 4	g. 2602C>T	p.Arg134Stop	p.Arg108Stop	Haematoma, haemarthrosis, gum bleeding, melena,umbilical cord bleeding, bruises
P3	FGA/exon 4	g. 3138C>T	p.Gln150Stop	p.Gln131Stop	Bruises, gum bleeding, haemarthrosis, umbilical cord bleeding, haematoma
P4	FGA/exon 5	g. 3807C>T	p.Arg178Stop	p.Arg159Stop	Bruises, gum bleeding, haemarthrosis, umbilical cord bleeding, haematoma
P5	FGA/exon 4	g. 3192C>T	p.Arg168Stop	p.Arg149Stop	Haematoma, bruises, bleeding,post minor surgery bleeding
P6	FGG/exon 2	g. 2018G>C (IVS2 + 1G>C)	-	-	Bruises, haematoma

Conclusions: We have reported six mutations leading to congenital afibrinogenemia in a cohort of 7 patients.Further studies of the association between phenotype and genotype in this subset of patients are needed to find the incidence of these rare bleeding disorders in our country.

FXIII08

Automated fibrin structure assay: determination of normal range

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Background: Fibers thickness of the fibrin clot plays an important role on the clot stability and its resistance to the fibrinolysis. An innovative concept, based on the relationship between light spectrum through the fibrin clot and its fiber nanostructure (C.Dassi et al., ISTH 15ABS-3593), thanks to a multi-wavelength STA^{â} prototype, allows real time determination of the fibrin structure (FS) in plasma.

Aims: To determine the precision, the normal range of the FS method in comparison with Thrombin Generation (TG) on CAT^{â} and discriminate patients from healthy volunteers (HV).

Methods: Fresh plasmas were collected from 102 HV (17 women - 85 men) and from 38 patients (12 women - 26 men). Plasmas were incubated with low Tissue Factor concentration and t-PA and triggered by Ca^{++} in FS method. The number of protofibrils (Np) and nanostructure parameters were measured/determined during 30 min at 37°C. TG was performed with the same reagents without t-PA. Precision was determined using Quality Control plasmas on 33 consecutive days. Normal range was determined on HV, using centile 2.5 and 97.5%. The most relevant parameters were used to discriminate patients from HV.

Results: The FS results showed a good precision (CV < 6% and < 8% for fibrin formation and lysis), about 2X less than TG. All the HV FS profiles were well distributed allowing the normal range calculation. These profiles were independent of the gender in the opposite of TG. 87% patient plasmas were discriminated in FS method both on Np within 10 and 30 min respectively. The temporal parameters were well correlated between both methods. The lack of correlation between Np and TG peak confirmed that FS method gives a different information from TG. Abnormal profiles could be clustered and need further investigations to be related with hemostasis disorders.

Conclusions: This new automated FS method provides new data on clot analysis and could be a useful tool in clinical practice in the management of hemostasis disorders.

FXIII09

Characterization of phenotypic expression of inherited fibrinogen coagulopathy in Pakistani index patients

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Background: Congenital afibrinogenemia is inherited as an autosomal recessive trait and consanguinity is common among affected families. Phenotypic expression of this particular factor deficiency is still not unanimously established in research literature globally as the disease manifests with variability in symptoms which may ranges from minimal bleeding to catastrophic hemorrhage.

Aims: To characterize the clinical manifestations of patients to set a standard parameter of bleeding severity in our local population.

Methods: This descriptive and cross sectional study was conducted at NIBD Karachi in collaboration with Chugtai's Lab and CHL Lahore in conformance with the Helsinki declaration. Consent was signed from all participants. Inclusion criteria for index patients specifically encompass the diagnosed cases of congenital afibrinogenemia excluding all acquired causes of this condition. Initially all the samples were processed at collection centers and platelet poor plasma and serum was separated and collected in labeled aliquots. Samples were transported to NIBD Karachi by maintaining cold chain. First line investigations including prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen levels by clauss method were performed to reconfirm the results. Grading of every individual's signs and symptoms depends upon severity of the disease and define here as per bleeding score [Tossetto et al.].

Results: Total 18 patients were evaluated with mean age group10 ± 2 yrs and 11 were males. Two patients were sibling. PT was >120s, APTT >180s and fibrinogen levels were (< 0.2 g/l).The most common symptom is umbilical bleeding (95%) followed by circumcision hemorrhage (90%) in males, cutaneous manifestations (85%) and epistaxis(68%).

Conclusions: The clinical manifestations of congenital afibrinogenemia in our local population are more or less same as reported in global literature. Few exceptions do exists as we didn't find most of the rare complications in our set of patients so far.

FXIII10

Functional/antigen fibrinogen ratio in different clinical situations

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Background: Inherited disorders of fibrinogen (Fg) are rare and can be divided into quantitative (a- and hypo) and qualitative (dys- and hypodys).

Aims: It was to analyse clinical and laboratory phenotypes of patients with Fg's defects.

Methods: Results of 44 individuals (30 women, 14 men, age range:1–87 year old) with low Fg (M.Clauss) (FgF), were retrospectively analysed. Fg antigen (M.Laurell) (FgAg) and the ratio (R) FgF/FgAg together with other laboratory results were also analysed. Clinical and laboratory background, familiar (FH) and personal (PH), was taken into account. Dysfibrinogenemia (dysFg) R cut off was estimated based on 26 controls.

Results: Controls: R (0.69–1.15) shows normal distribution ($p_{D'Agostino\text{-}Pearson}$=0.30) with mean value of 0.94 (SD=0.14). $R_{lower\ limit}$ was 0.67 (mean value-2SD; CI 95%).

Patients showed FgF (not detectable-195 mg/dL) under the reference value (200–400 mg/dL). Results were compatible with dysFg (R:0.19–0.66; FgF:52–170 mg/dL) in 31.82% (14/44) of patients; the rest (30/44) had R ≥ 0.67 (R:0.71–1.08; FgF:not detectable-195 mg/dL). Table shows laboratory and clinical (asymptomatic, bleeding, stroke or obstetric complications) backgrounds; thrombin time (TT) behaviour in patients plasma and mixing tests is also displayed.

Other abnormal tests as minor defects of platelet function (9), low FVIII (5, 1/5 < 15UI/dL), low platelet count (2, 1/2 < $50x10^9$/L) and possible VWD (1), were detected in some patients with FH and/or PH of bleeding (6/9 in R < 0.67, and 11/18 in R ≥ 0.67). None of asymptomatic patients or those with stroke or obstetric complications showed additional abnormal tests.

Table (Abstract FXIII10)

R < 0.67; FgFrange=52–170 mg/dL; N = 14 patients			R ≥ 0.67; FgFrange=ND-195 mg/dL; N = 30 patients		
Patients	Family History	Personal History	Patients	Family History	Personal History
5	Abnormal Fg	Bleeding (4) Foetal loss (1)	7	Bleeding	Bleeding
1	Bleeding	Bleeding	11	No	Bleeding
8	No	Bleeding (4) Stroke (2) Foetal loss + Abortion <10 wk (1) Abortion<10 wk (1)	8	No	Foetal loss (4) Foetal loss + Abortion<10 wk (1) Abortion<10 wk(3)
–	–	–	4	No	Asymptomatic (haematological disease 2/4)
Patients	TT (FgF range)	TT mixing test correction 1P:1N	Patients	TT (FgF range)	TT mixing test correction 1P:1N
10	Prolonged (52–154 mg/dL)	Yes (1), No (9)	4	Prolonged (ND-195 mg/dL)	Yes (3), No (1)
4	Normal (110–170 mg/dL)	–	26	Normal (120–195 mg/dL)	–

Conclusions: These results suggest that R assessment together with TT and its mixing test with normal plasma can be useful tools to suspect qualitative (no-acquired causes, R < 0.67, prolonged TT/not corrected by normal plasma) or quantitative (no-acquired causes, R ≥ 0.67; prolonged TT/corrected by normal plasma) Fg alterations associated with haemorrhagic, thrombotic or obstetric complications.

FXIII11

Molecular modelling of identified six novel missense mutations in pakistani congenital afibrinogenemia patients

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Background: Congenital afibrinogenemia (OMIM #202400) is a rare coagulation disorder which was first described in 1920. It is transmitted as an autosomal recessive trait characterized by absent levels of fibrinogen (factor I) in plasma. Consanguinity in Pakistan and its neighboring countries has resulted in higher cases of congenital fibrinogen deficiency in their respective populations.
Aims: This study focuses on the detection of mutations in the fibrinogen genes by DNA sequencing and molecular modeling of missense mutations in all three genes (Fibrinogen gene alpha (FGA), beta (FGB) and gamma (FGG) in Pakistani patients.
Methods: This descriptive and cross sectional study was conducted in Karachi and Lahore and fully complied with the Declaration of Helsinki. Patients with fibrinogen deficiency (tested by Fibrinogen functional assay from Laboratoire Stago, Asnieres, France) were screened for mutations in the Fibrinogen gene alpha (FGA), beta (FGB) and gamma (FGG) genes by direct sequencing. Molecular modeling was performed to predict the putative structure functional impact of the missense mutations identified in this study.
Results: Out of thirteen 4 patients had missense mutations in FGA and two had missense mutation in FGB. The missense mutations are predicted to result in loss of stability since they a) break ordered regions b) cause clashes in the hydrophobic core of the protein.
Conclusions: Congenital afibrinogenemia is a rapid growing problem in countries such as Pakistan where consanguinity is frequently practiced. This study illustrates the fact that mutations in FGA are relatively more common in our population than those in FGB where as FGG mutations appear rarer.

Genomics in Thrombosis and Hemostasis

GEN01

Diagnosis of inherited platelet disorders by a panel-based next generation sequencing approach

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Background: Despite extensive platelet function analysis, many patients with bleeding disorders remain without substantiated diagnosis. However, correct classification of the underlying genetic cause is essential for optimal treatment and genetic counseling.
Aims: We developed a panel-based next generation sequencing (NGS) approach to identify genetic variants in patients suspected to have inherited platelet disorders (IPD).
Methods: 59 genes known to be involved in platelet biogenesis or function were selected. We designed 6,800 probes that cover all exons and additional regulatory sequences. DNA samples from 36 patients were processed by MiSeq Sequencing System by Illumina. Common SNPs were excluded from further data analysis by a default filter. DNA from five patients with previously characterized genetic defects was used for quality control and process validation. The study was conducted in accordance with local Institutional Review Board guidelines.
Results: We readily confirmed all five of the previously identified mutations in the quality control samples validating the results from our approach. Four out of 31 samples with a so far unidentified genetic cause failed quality control and did not provide any result. 15 out of the remaining 27 samples revealed one or two unclassified genetic variants of the tested 59 genes. As some target regions can have low coverage or single gaps, we monitored read coverage for each patient. So far, we were able to confirm any NGS-detected mutation by Sanger sequencing. In one case of a novel likely pathogenic variant, segregation analysis ruled out that the mutation was associated with the phenotype.
Conclusions: Here, we developed a NGS-panel that is sensitive enough to identify known and unknown genetic variants in platelet-specific genes of classified and unclassified patients. The authors consider this panel capable of expanding genetic testing to patients that have failed standard approach to diagnosis following published guidelines.

GEN02

Design and application of a twenty-three-gene panel by next-generation sequencing for inherited coagulation bleeding disorders

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Background: Until recently, Molecular testing of Inherited bleeding coagulation disorders (IBCDs) has been mainly performed by Sanger sequencing, a technique known to be time consuming and expensive. Currently, Next-Generation Sequencing (NGS) offers a new potential approach that enables the simultaneous investigation of multiple genes at manageable cost.
Aims: To design and to analyze the applicability of a 23-gene NGS panel in the molecular diagnosis of patients with IBCDs.
Methods: We enrolled a total of 20 patients with an IBCDs phenotype who were studied using NGS technology. A custom target enrichment library was designed to capture twenty-three genes known to be associated with IBCDs. Probes were generated for 296 targets to cover 86.3 kb regions (all exons and flanking regions) of these genes. Sanger sequencing was performed to validate all causative variants identified by NGS.
Results: The use of this 23-gene panel approach allowed us to identify the causative variants of the IBCDs in all patients. Overall, Twenty-onepathogenic variants were found, including six novel mutationsaffecting *F8, FGA, F11, F10* and *VWF* genes and fifteen previously reported variants were detected. Of the 21 alterations, 18 were missense and 3 were frameshift changes due to microdeletions. NGS and Sanger sequencing were 100% concordant.
Conclusions: Inherited coagulation disorders could be successfully molecularly characterized by using our 23-gene Next Generation DNA Sequencing panel. Our results demonstrate that this approach could be an accurate, reproducible, and reliable tool in the rapid genetic diagnosis of IBCDs.

GEN03

Utilizing the strength of pedigree-based study design to identify novel tPA Loci

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Background: Tissue Plasminogen Activator (tPA) is a serine protease that mediates the conversion of plasminogen to plasmin, the major enzyme responsible for endogenous fibrinolysis. tPA plasma levels are highly heritable. A mega-GWAS implicated 3 genes involved in tPA regulation, which collectively explain only ~1% of tPA variance. Rare genetic variants with large effect size could underlie the unaccounted tPA variation. A pedigree-based study in a founder population is an optimal design to test for co-segregation of putative rare variants.
Aims: To identify rare genetic variants associated with tPA plasma levels.
Methods: The study sample consists of 178 individuals from 4 extended French-Canadian pedigrees ascertained on a proband with venous thromboembolism. We used a bayesian oligogenic joint linkage and segregation analysis to conduct a genome-wide linkage scan of microsatellite markers with tPA levels, followed by a variance-component approach to validate findings. An association analysis, using a linear mixed regression model adjusted for relatedness and covariates was used, to test for association between SNPs and tPA levels in the identified genomic region. The significance threshold was determined by estimating the effective number of tests, accounting for the LD structure.
Results: Both linkage analysis approaches identified a genome-wide significant signal on chromosome 11q14 region (LOD score=3.51; log10(BF)=2.56, p = 0.001). There were 3,286 genotyped SNPs in the linkage region common to both approaches, corresponding to a significance threshold of $2.89e^{-5}$. Nine SNPs met the significance threshold. Imputed SNP data in the region was analyzed for refinement, which identified the strongest associations with rare variants in this region (top imputed SNP: p = $3.48e^{-7}$, MAF=0.11 vs to MAF=0.039 in population).
Conclusions: Linkage analysis followed by genetic association testing in pedigrees identified a novel region of interest for tPA variation, providing new insights on tPA determinants.

GEN04

ThromboGenomics: a comprehensive high-throughput sequencing test for the diagnosis of inherited bleeding, thrombotic and platelet disorders

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Background: Inherited bleeding, thrombotic and platelet disorders (BPDs) are rare diseases affecting approximately 300 individuals per million births. With the exception of haemophilia and von Willebrand disease, molecular analysis for BPD patients is often unavailable. If possible, genetic testing is routinely performed in a step-wise manner using Sanger Sequencing. This approach causes significant

delays and a conclusive molecular diagnosis is often never reached, compromising treatment and impeding the rapid identification of affected relatives.

Aims: To address this unmet diagnostic need, we designed a high-throughput sequencing platform targeting 63 BPD disease genes.

Methods: The platform can identify single nucleotide variants, short insertions/deletions and large copy number variants (though not inversions), which are subjected to automated filtering for diagnostic prioritisation, resulting in an average of only 5.34 candidate variants per individual. We sequenced 159 and 141 patients respectively from individuals with and without previously known causal variants for BPD. Among the latter group, 61 patients had phenotypes strongly indicative of a particular molecular aetiology while the remainder had an aetiology that was *a priori* highly uncertain.

Results: All samples of patients with previously known causal variants were recapitulated and, when the aetiology was suspected but unknown, a molecular diagnosis was reached in 56 of 61 cases.

Research will be presented for a new version of the ThromboGenomics platform (TG2.0, 74 genes), including analysis of a large Thalassemia and Hereditary Hemorrhagic Telangiectasia cohort of over 200 patients.

Conclusions: The ThromboGenomics platform provides a comprehensive and affordable DNA-based test to diagnose patients suspected of having a known inherited BPD.

GEN05

Improved venous thromboembolism risk assessment with a multilocus genetic risk score

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Background: In the development of venous thromboembolism (VTE) genetics contribute in a relevant manner. In clinical routine the presence of two mutations Factor V Leiden (FVL) and G20210A Prothrombin (PT) are analysed to evaluate this genetic contribution. However, the sensitivity of FVL and PT is very low. New algorithms combining clinical data with genetic variables like TiC have demonstrated higher sensitivity.

Aims: To evaluate whether the use of Genetic Risk Scores (TiC) provides a better assessment of the VTE risk than a model based only on FVL-PT in a population of French patients with unprovoked idiopathic VTE.

Methods: The VTE predictive capacity of FVL+PT and TIC panels were compared. For each panel a multi-locus GRS was computed for each individual as the sum of the number of risk alleles, after weighting them by its effect size. A population of 103 idiopathic unprovoked VTE (35 males, 68 females; 47.5 ± 13.9 years old) and 248 controls (109 males, 139 females; 49.0 ± 14.9 years old) was used.

The predictive capacity was assessed by calculating the c-statistic (AUC-ROC); sensitivity, specificity Positive and Negative Likehoood ratios and OR of a positive test result. Informed consent was obtained and the studies were approved by recognised ethics committees.

Results: When compared to FVL+PT, the use of TIC panel significantly improved the capacity to discriminate VTE (AUC-ROC: 0.613 vs 0.712, p = 0.01). Moreover, clinical sensitivity (number of cases where the score of the panel was higher than the cut-off) increases very significantly in relation to FVL+PT (21.36% vs 96.12%, $P < 0.001$). Specificity was higher with FVL+PT (93.55 vs 59.68, $P < 0.001$).

Table

Variant (presence)	Controls (%)	Cases (%)	p value	OR	+Likehood ratio (p = 0.7)	-Likehood ratio (p = 0.001)
FVL (rs6025)	2.02	1.94	0.7			
F2 (rs17999639)	2.82	4.85	0.5			
AB0-A1	35.7	31.07	0.4			
F12 (rs1801020)	2.02	5.82	0.12			
F13 (rs5985)	56.5	92.23	<0.0001			
SERPIN A10 (rs22232698)	1.61	0	0.45			
SERPIN C1 (rs121909548)	0.40	0.97	0.89			
TiC panel				17.71	2.38	0.06
FVL+PT panel				3.71	3.31	0.84

Conclusions: TIC panel significantly improves the predictive capacity of VTE risk when compared to FVL+PT. Thus, our study suggests that the use of algorithms with a set of confirmed susceptibility loci (TIC) improves disease risk assessment and could be also an aid in the prevention, diagnosis and treatment of VTE disease.

GEN06

Identifying the genetic basis of rare bleeding and platelet phenotypes using systematic phenotyping and genome sequencing

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Background: The majority of rare BPD do not have an identified genetic basis. Whilst whole genomic sequencing is now an affordable approach, small family sizes, variable penetrance and phenotypic variability are barriers to identifying the responsible genetic variants. We have used a systematised phenotyping approach combined with novel clustering analyses to detect implicated genes and candidate causal mutations from next generation sequencing (NGS).

Aims: Detection of novel genetic causes of bleeding and platelet phenotypes.

Methods: 848/1247 index cases and 78/87 affected relatives have been sequenced/phenotyped. The Human Phenotype Ontology (HPO) has been expanded to better capture bleeding phenotype and laboratory data. New clustering algorithms have been developed to group patients with similar phenotypes and a presumed similar genetic basis.

Results: In 115 cases a definitive or likely genetic explanation has been identified and in 13 a partial genetic explanation. Four novel genes responsible for platelet abnormalities including *DIAPH1*, *SRC* and *TRPM7* have been identified and unsuspected variants of previously known syndromes including *MYH9* syndromes and HPS have been revealed. Finally we have shown that large numbers of cases are explained by variants in known genes e.g. 27 by *ACTN1* variants and 8 by *ANKRD26* variants. In total 43 genes have been identified as known or possible explanations and are under further investigation.

Conclusions: A systematic method of detailed phenotyping has been used as the basis for clustering analysis to group patients with putative similar genetic basis. This has then been combined with the filtered results of NGS to successfully identify novel genes and confirm previous candidate genes responsible for BPD. Syndromic phenotypes have been better defined and a large number of candidate genes remain to be explored. Moving from WES to WGS will now allow these methods to be extended to the non-coding space.

GEN07

FVII deficiency and thrombosis, coincidence or new mechanism of thrombophilia

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Background: A large number of arterial and venous thromboembolic events were reported in hemorragic coagulopathies such as in hemophilia A and B, Von Willebrand disease and factor VII deficiency.
Aims: We propose in this presentation a review of literature and a case report of a FVII deficiency associated with recurrent thrombosis.
Methods: We report a 34-year-old male patient with personal history of recurrent phlebitis and cerebral venous thrombosis. The investigations revealed only a prolonged prothrombin time (18''/12'') without any abnormalities of the thrombophilia testing, no inhibitors deficiency, no lupus anticoagulants, no increase level of FVIII c, FV and FII polymorphisms are negatives. Further investigations revealed in prothrombin complex testing an isolated FVII deficiency at 26%.
Results: The direct sequencing of F 7 performed in the department of Biologic Hematology - CHU Montpellier - France, revealed a homozygous mutation "C.-56C>T ".It is a mutation of the promoter, never reported in the databases and located near the transcription factor binding site HNF4. The *in Silico* analysis have confirmed the asymptomatic character of our patient (absence of hemorrhage) but the implication of these mutation in thrombotic events remains to be elucidated. The search of an eventual resistance to the TFPI will be achieved.The absence of bleeding history, led us to treat this patient with oral anticoagulant vitamin k antagonist targeting INR values between $2 < INR < 3$.
Conclusions: The use of oral anticoagulation prophylaxis in patients with coexistence of FVII deficiency and thrombosis can be safe and well tolerated, especially in asymptomatic cases. The question of whether some F7 genotypes are linked to a thrombotic phenotype remains unanswered and will require the evaluation of a larger series of FVII-deficient patients with venous thrombosis.

GEN08

Identification of molecular defects in ITGA2B and ITGB3 genes and phenotypic correlation in Pakistani patients with glanzmann thrombasthenia

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Background: Glanzmann thrombasthenia (GT) is the most common inherited platelet functional defect. It is an autosomal recessive disorder, characterized by a bleeding diathesis. Incidence is increased in those geographical locations where consanguineous marriages are common. The defect is caused by mutations in the genes encoding ITGA2B or ITGB3 located on chromosome 17. This defect results in qualitative or quantitative abnormalities of the fibrinogen receptor, αIIb-β3 integrin found on platelets.
Aims: The aim of this study was to identify and correlate the mutations in GT patients with phenotype of the patient.
Methods: 20 patients with GT were enrolled in the study after obtaining informed consent. History was recorded in a structured data sheet and bleeding tendency was assessed using bleeding score (BS) questionnaire. Platelet counts were performed along with morphology. Prothrombin Time, Activated Partial Thromboplastin Time and Fibrinogen levels were done initially. Platelet aggregation studies were done using agonists (ADP, Collagen, Adrenaline and Ristocetin). Flowcytometry was performed on BD FACSCALIBUR (using CD 41, 61, 42a, 42b antibodies *BD Biosciences*) to determine the expression of platelet integrin αIIbβ3 in GT patients. Mutational analysis was done by Sanger sequencing using automated genetic analyzer ABI 3500 (*Applied biosystems*).
Results: Mutations were identified in 11 patients. Missense mutations were seen in most of the GT patients. The remaining mutations were heterogeneous and were distributed throughout the length of the gene. Genetic Analysis was not done on five related patients.
Conclusions: Severe type I GT was the most common defect found in this study. Carrier detection and genetic counseling in affected families is an important step for decreasing the burden of severe disease and may limit its spread.

GEN10

A novel nonsense mutation Glu326* of *PROS1* Leading to ProS Deficiency

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Background: Protein S (ProS), a physical anticoagulant in plasma, plays a crucial role in the regulation of blood coagulation. ProS involves in the inactivation of factor Va and factor VIIIa, in addition to thrombin and tissue factor. Inherited Pro S deficiency (PSD), which confers a 2.5- to 11- fold increased risk of venous thrombosis (VT), is an autosomal disorder and correlated with genetic defect of ProS encoding gene (*PROS1*). Hundreds of *PROS1* mutations have been reported, indicating that PSD is genetically heterogenous.
Aims: In this study, a pedigree of hereditary PSD was investigated and a novel nonsense mutation in *PROS1* was identified.
Methods: Subjects included in this study were the proband, his father and sister. The proband was a 24-year old Chinese man and suffered from recurrent VT. Acquired risk factors such as tumor, immobility, hormone-replacement therapy were not identified. No other family members had a definite history of VT except his uncle. Peripheral blood samples were collected and screened for the activity of Protein C, ProS, and antithrombin. Genomic DNA was extracted for exemplification and sequencing of *PROS1* and the VT associated inherited risk factors (*PROC* c.565C>T, *PROC* c.574_576del, and *THBD* c.-151G>T).
Results: The proband had a decreased ProS activity of 35% (reference range, 73–150%). The protein C and antithrombin activity were within the reference ranges (115 and 94%, respectively). Genetic test indicated that the proband was heterozygous for a G to T nonsense mutation at the position 976 in exon 10 (c.976 G>T) of *PROS1,* generating a premature stop codon 326 (p.Glu326*). Other common genetic risk factors were unidentified. The father and sister were also detected as PSD, with a reduced ProS activity of 23% and 21% respectively, and with the same gene mutation. His uncle was unavailable for thrombophilia test.
Conclusions: We identify a novel nonsense mutation Glu326* of *PROS1* that causes ProS deficiency. However, its association with VT requires further investigation.

GEN11

Identification of two novel single nucleotide variants of the complement Factor H (*CFH*) and Factor I (*CFI*) genes in a familial form of atypical hemolytic uremic syndrome (aHUS)

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Background: Atypical hemolytic uremic syndrome (aHUS) is characterized by microangiopathic hemolytic anemia, thrombocytopenia and acute renal failure. Most aHUS cases involve sequence variations in genes encoding complement proteins.

Aims: A 27-year-old woman with an episode of thrombotic microangiopathy 1 month post-partum presented with severe anemia (hematocrit: 22%, hemoglobin: 7.6 g/dL), thrombocytopenia (138 000/μL), elevated LDH (654 IU/L) and deteriorating renal function (creatinine: 3.3 mg/dL); she and her available relatives were screened for mutations/polymorphisms in aHUS-associated complement genes.

Methods: After extracting gDNA from whole blood (Wizard® Genomic DNA Purification Kit, Promega), PCR products of coding sequences and intronic flanking regions of complement genes were sequenced by ABI PRISM 310 Genetic Analyzer (Applied Biosystems). *In silico* analysis for pathogenicity was completed with *Polyphen2-HDIV*, *PhyloP*/*Phastcons* (*MutationTaster*), *SIFT* and *PANTHER*. All the participants provided informed written consent.

Results: The patient was diagnosed with aHUS (all ADAMTS13 parameters were normal). Comprehensive screening of aHUS-associated complement genes identified two novel single nucleotide variants: *CFH* c.575G>A, p.C192Y (exon 5) (NM_000186), predicted to be pathogenic by 4 of 5 available pathogenicity prediction programs; and *CFI* c.1189G>T, p.V397L (exon 11) (NM_000204), predicted pathogenic by 0 of 6 available pathogenicity prediction programs. Figure 1 shows the segregation of these variants in the pedigree. The subjects are heterozygous for the identified variants.

Conclusions: We identified two novel genetic variants in the *CFH* and *CFI* genes in a patient with aHUS, who inherited one variant from each parent. Although the *CFI* variant is predicted to be benign, the *CFH* variant is predicted to be damaging. It is located in exon 5, which encodes a portion of the factor H protein implicated in binding to C3b.

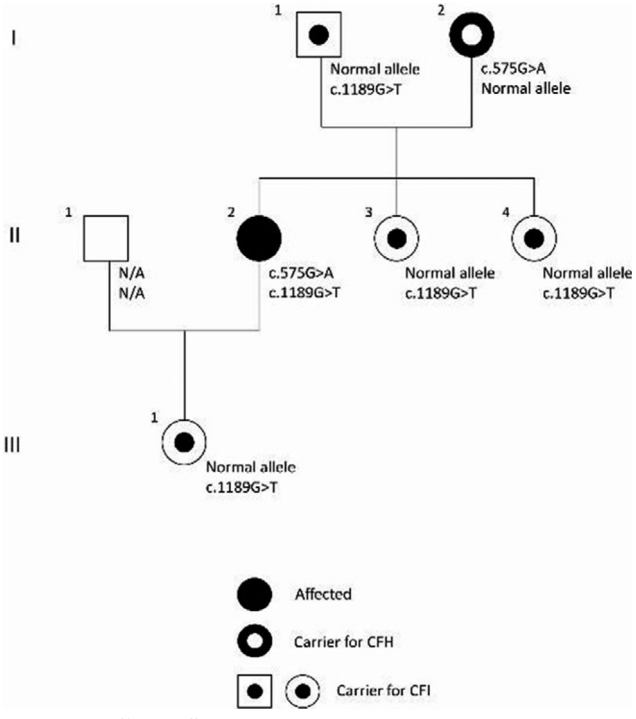

Figure 1 Family's pedigree.

GEN12

High prevalence of VKORC1*3 (G9041A) genetic polymorphism in north indians: a study on patients with cardiac disorders on acenocoumarol

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Background: Coumarin derivatives such as warfarin and acenocoumarol are used in various disorders such as deep venous thrombosis, pulmonary embolism, atrial fibrillation and artificial heart valves. They have improved prognosis of patients with thromboembolic disease. An individual′s response to coumarins depends on several factors. The non-genetic factors include age, gender, body mass index, diet and interacting drugs. Among the genetic factors, the cytochrome P450 system and vitamin K epoxide reductase complex subunit 1 play a key role in drug metabolism.

Aims: This was a prospective hospital based study in which allele and genotypic frequencies of CYP2C9 gene polymorphisms; 430C>T and 1075A>C and VKORC1 gene polymorphisms; 1639G>A, 9041G>A and 6009C>T in 106 alleles of north Indian patients with valve replacement on acenocoumarol were determined and their effect on acenocoumarol dosing was studied.

Methods: Genomic DNA was extracted from peripheral blood leucocytes using midi-kit method. Two sets of primers sequences, forward primer and reverse primer were designed for *CYP2C9**2 (C430T, exon 3) and *CYP2C9*3* (A1075C, exon 7) analysis and for *VKORC1*2* (G3673A), *VKORC1*3* (G9041A) and *VKORC1*4* (C6009T) analysis. Different PCR products and the lengths of the different fragments were generated by PCR-RFLP method.

Results: In 53 patients with valve replacement on acenocoumarol with stable INR, the allele frequency of CYP2C9*2 and CYP2C9*3 gene polymorphisms was 0.05 and 0.17 respectively and that of VKORC1 *2,*3 and *4 gene polymorphisms was 0.15, 0.72 and 0.11 respectively. The presence of CYP2C9*3 orVKORC1*2 gene polymorphism were associated with decrease in acenocoumarol dose requirements (p values 0.03 and 0.02 respectively).

Conclusions: This study confirmed the association of lower mean weekly dosages of acenocoumarol in patients with CYP2C9*3 and VKORC1*2gene polymorphisms. An unusually high frequency of 9041A polymorphism in VKORC1 was found in study population.

GEN13

Clinical profile of patients with proximal deep venous thrombosis patients - a prospective observational study from a tertiary care hospital in North India

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Background: Deep Venous Thrombosis (DVT) is a serious medical problem which results in considerable morbidity as well as mortality.
Aims: Risk factors associated with proximal DVT.
Methods: Adult patients with symptomatic first episode of deep venous thrombosis of the proximal veins of lower limbs (femoral and popliteal veins) over 12 months from June 2013 to June 2014 were enrolled in the study. Venous thrombosis was diagnosed by duplex ultrasonography. Anticoagulation with heparin (either unfractionated heparin or LMWH) along with vitamin K antagonist (warfarin) was initiated from the first treatment day following confirmation of diagnosis. Heparin was stopped when an INR of >2 was maintained for at least 48 h and thereafter warfarin was continued for at least 6 months. Serial monitoring of INR was done to maintain an INR value of about 2.5 (desirable range, 2.0–3.0).
Results: A total of 100 patients, with 113 thrombotic events were recorded during the study period. Acute unilateral DVT was observed in 87 patients with right lower limb being involved in 27 patients and the left leg in 60 patients.
The mean age of the enrolled patients was 39.9 $\pm$ 12.025 years with 55% being males.
Risk factors for predisposition for DVT were identified in 57 patients. These included primary thrombophilia (21%), pregnancy/postpartum period (19%), prolonged bed rest/immobility (57%) and underlying malignancy (3%). Among the patients with an underlying thrombophillic state, antiphospholipid antibodies(11),Factor V leiden mutation(5),Protein C and S deficiencies (2 patients each). One patient had an underlying Antithrombin III deficiency.
Local swelling (92.3%) was the most common presenting complaint, followed by pain (91.2%) and erythema at the local site in 89%. 18% of the patients had a history recurrent of DVT in the past among which 14% had an underlying predisposition factor for DVT.
Conclusions: Primary thrombophilia remains remains an important predisposing factor for patients with deep venous thrombosis.

Hemostasis & Malignancy

HEM01

Aspirin inhibits colon cancer cell proliferation through synergistic suppression of NF-*k*B and c-MYC oncoproteins

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Background: Clinical trials, epidemiological and both *in vitro* and *in vivo* experimental studies have provided rationale for the use of aspirin as an agent for the treatment and prevention of colorectal neoplasia. Yet, the molecular mechanisms underlying the chemopreventive efficacy of aspirin remain ill-defined, while aspirin is currently not recommended for colorectal cancer prevention.
Aims: The goal of this study was to characterize the molecular mechanisms by which aspirin directly inhibits colon cancer cell proliferation. In addition, we also investigated the intriguing possibility that aspirin may reduce the procoagulant activity of cancer cells by changing the expression and activity of tissue factor (TF) on cancer cells.
Methods: We used an isogenic pair of colon cancer cell lines, SW480 (nonmetastatic) and SW620 (metastatic). After treatment with either 2.5 or 5 mM aspirin for 48 h, the following three assays were performed: cell proliferation was measured by Cell Titer 96® AQueous One Solution Proliferation Assay; TF activity was quantified by FXa Chromogenic Assay; changes in the expression levels of c-MYC, COX-2, NF-*k*B and TF were assessed by western blotting.
Results: We demonstrated that anti-inflammatory doses of aspirin inhibit cancer cell proliferation by synergistically limiting the nuclear translocation of the transcription factor NF-*k*B and by downregulating the expression of the oncoprotein c-MYC. In addition, we found that aspirin inhibits the procoagulant activity of colon cancer cells, suggesting a potential additional anti-thrombotic effect for aspirin.
Conclusions: Our data suggests that the mild anti-cancer effect of aspirin may not be solely a consequence of the inhibition of a standalone target, but due to combinatorial changes in the localization of the transcription factor NF-*k*B and through regulation of expression of proteins key to cancer progression, including the oncoprotein c-MYC and the procoagulant protein TF.

HEM02

Platelets upregulate c-MYC oncoprotein in pancreatic cancer cells: potential mechanism of platelet-driven neoplastic proliferation

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Background: The oncoprotein c-MYC, which governs neoplastic proliferation, is overexpressed in pancreatic tumors at the metastatic niche as compared to primary tumors, with the overexpression of c-MYC indicative of a poor clinical outcome for patients with cancer. At present, the oncogenic pathways mediating c-MYC upregulation in metastatic cancer cells are largely unknown. Our exciting preliminary data demonstrate for the first time that platelets upregulate c-MYC protein levels in PANC-1 pancreatic cancer cells, suggesting a novel prometastatic role for platelets in pancreatic cancer.
Aims: Define the mechanism by which platelets upregulate c-MYC protein levels in pancreatic cancer cells.
Methods: We utilized a co-culture platform to examine the effect of resting and thrombin-stimulated human platelets on c-MYC expression in PANC-1 cancer cells. Platelet inhibitors, including the $\alpha_{IIb}\beta_3$ blocker Integrilin, were used to study the molecular mechanisms by which platelets upregulate c-MYC expression in cancer cells. Changes in c-MYC oncoprotein were detected by Western Blotting. The physical association between platelets and PANC-1 cancer cells following co-culture was quantified by immunoblotting with the platelet-specific anti-α_{IIb} antibody.
Results: Resting and thrombin-activated platelets induced an upregulation of c-MYC expression in PANC-1 cancer cells. The effect of platelets on c-MYC expression was largely dependent on direct contact with the cancer cells, as the preincubation of platelets with Integrilin reduced platelet-induced c-MYC expression.
Conclusions: We show that the expression of c-MYC in PANC-1 cancer cells can be upregulated by platelets, suggesting that the overexpression of c-MYC at the metastatic niche may be inhibited through the use of platelet inhibitors. We are currently characterizing the molecular underpinnings of platelet-mediated c-MYC regulation with respect to the contribution of platelets to cancer cell proliferation and growth.

HEM03

Optimal doses of tinzaparin to reduce both cancer-associated thrombosis and tumor growth in a mouse model of ectopic pancreatic syngeneic tumor

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Background: Thromboprophylaxis with low-molecular-weight heparins (LMWH) was previously demonstrated to reduce venous thromboembolism risk and improve outcomes in cancer patients. Moreover, preclinical models suggest that LMWH may offer additional benefits through direct antitumor properties. To date, the optimal doses of LMWH that may prevent both cancer-related thrombosis and tumor development are unknown.
Aims: To determine the tinzaparin optimal doses preventing both cancer-related thrombosis and tumor development in a pancreatic cancer syngeneic ectopic model.
Methods: The tinzaparin optimal doses to generate a plasma anti-Xa activity > 0.2 IU/mL were determined *in vivo* in wild type (WT) mice. A syngeneic ectopic model of cancer was induced in WT mice using the mouse pancreatic cancer cell line Panc02. Mice were injected daily with 200, 300, 400 IU/kg tinzaparin or placebo from day 8 to 25 following tumor induction. Kinetics of thrombus formation and fibrin generation were determined by digital real time intravital microscopy. Tumor growth and bleeding times were measured and compared in the different groups.
Results: Doses ranging from 0 to 150 IU/kg generated plasma anti-Xa levels< 0.2 IU/mL, whereas doses >200 IU/kg generated anti-Xa activities > 0.2 IU/mL. At day 25 following tumor induction, as compared to controls, kinetics of thrombus formation were not affected in mice treated with 200 IU/kg. On the contrary, it was strongly affected in mice treated with 300 & 400 IU/kg. Interestingly, tumor growth was significantly decreased in mice treated with 200, 300 & 400 IU/kg tinzaparin as compared to controls, with no significant difference between these tinzaparin groups. Bleeding times were significantly increased only in mice treated with 300 & 400 IU/kg as compared to controls.
Conclusions: Daily 300 & 400 IU/kg tinzaparin treatment decreases both cancer-related thrombus formation and tumor growth, but at the price of a significant increase in bleeding times.

HEM05

A comparison of cancer types and the epidemiology of first venous thromboembolism in patients with active disease

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Background: Population studies on the epidemiology of venous thromboembolism (VTE) in patients with active cancer are limited. The frequency of cancer in VTE cohorts has been well described but there are no published data for the incidence rates (IR) for VTE among patients with active cancer stratified by cancer type.
Aims: We aimed to use a population based observational cohort study approach to estimate the incidence of first VTE in patients with active cancer stratified by subgroups.
Methods: A cohort study was undertaken with the source population consisting of all patients in the UK Clinical Practice Research Datalink, with additional linked information on hospitalisations and cause of death. The 90 days before and after a cancer-related clinical diagnosis or therapy defined the period of an active cancer (at-risk period). Between 2001 and 2011 all first VTE events during the at-risk period were identified using a validated algorithm. IR of first cancer-associated VTE were provided by age and cancer type.
Results: In a total of 139,467 patients with cancer contributing 112,738 active cancer-associated person-years of observation, 6592 incident VTEs, 46.3% DVTs and 53.7% PE with or without DVT, were observed. The mean age of VTE was 68.8 years. The most common types of cancer among VTE cases were prostate cancer in men (18.2%), breast cancer in women (16.7%), lung cancer (14.7%) and

Table Cancer type and Incidence Rates (95% CI) I. (Abstract HEM05)

Age	Bladder	Breast	Colon	Lung	Prostate
<18	0.0 (0.0–85.7)	0.0 (0.0–267.9)	0.0 (0.0–115.0)	0.0 (0.0–119.8)	0.0 (0.0–109.4)
18–29	9.0 (0.2–50.1)	0.0 (0.0–4.9)	2.2 (0.1–12.0)	7.1 (0.2–39.7)	0.0 (0.0–135.4)
30–39	1.7 (0.0–9.5)	2.6 (1.5–4.0)	7.4 (3.2–14.6)	11.2 (4.1–24.3)	0.0 (0.0–84.3)
40–49	4.4 (2.1–8.0)	2.3 (1.8–2.9)	7.4 (5.2–10.3)	9.3 (6.0–13.6)	1.9 (0.0–10.4)
50–59	2.9 (1.9–4.3)	2.7 (2.3–3.3)	5.9 (4.8–7.2)	11.4 (9.6–13.5)	2.8 (1.7–4.3)
60–69	3.3 (2.6–4.0)	3.9 (3.4–4.6)	7.2 (6.3–8.2)	10.8 (9.6–12.1)	4.2 (3.5–5.0)
70–79	4.0 (3.4–4.6)	5.1 (4.3–6.1)	8.3 (7.5–9.2)	9.8 (8.8–11.0)	4.3 (3.7–4.9)
80–89	2.9 (2.4–3.6)	5.8 (4.7–7.1)	6.7 (5.7–7.8)	9.0 (7.6–10.5)	4.2 (3.6–4.9)
>89	2.9 (1.6–4.9)	6.2 (4.0–9.2)	7.5 (5.0–11.0)	6.7 (3.6–11.5)	4.5 (3.0–6.5)

Table Cancer type and Incidence Rates (95% CI) II. (Abstract HEM05)

Age	Uterus	Hematologic	Brain	Ovary	Pancreas	Stomach
<18	–	0.7 (0.3–1.5)	0.7 (0.1–2.5)	0.0 (0.0–32.8)	0.0 (0.0–212.9)	0.0 (0.0–138.9)
18–29	0.0 (0.0–526.3)	1.5 (0.6–3.1)	0.9 (0.0–5.1)	5.4 (0.6–19.3)	0.0 (0.0–238.9)	11.7 (0.3–65.2)
30–39	0.0 (0.0–19.1)	3.2 (1.8–5.1)	5.1 (2.0–10.4)	8.7 (3.5–18.0)	22.4 (6.1–57.3)	8.2 (1.0–29.4)
40–49	5.7 (2.1–12.5)	2.7 (1.9–3.9)	10.7 (6.9–15.9)	7.8 (4.6–12.3)	15.9 (7.9–28.4)	12.9 (6.7–22.6)
50–59	6.0 (3.8–8.9)	4.6 (3.8–5.6)	14.0 (10.0–19.2)	9.2 (6.9–11.9)	20.7 (15.5–27.0)	12.8 (8.8–18.1)
60–69	8.0 (6.0–10.5)	4.7 (4.0–5.4)	16.1 (12.1–21.0)	12.2 (10.1–14.7)	16.3 (12.9–20.3)	10.9 (8.3–14.1)
70–79	7.6 (5.5–10.2)	4.4 (3.8–5.1)	14.1 (9.7–19.7)	13.0 (10.5–15.9)	11.7 (9.1–14.7)	11.8 (9.6–14.3)
80–89	9.3 (6.2–13.4)	5.0 (4.1–5.9)	6.6 (2.4–14.5)	15.6 (11.4–20.9)	12.2 (8.9–16.3)	6.6 (4.6–9.0)
>89	0.0 (0.0–10.6)	2.5 (1.2–4.8)	0.0 (0.0–35.9)	23.6 (8.7–51.4)	3.1 (0.4–11.2)	4.1 (1.1–10.5)

colon cancer (13.5%). IRs of VTE were 5.8 (CI 5.7–6.0) per 100 person years, Brain, lung, ovary, pancreas and stomach cancers had the highest incidence rates for VTE; bladder and breat cancer had the lowest incidence rates, see Table.
Conclusions: Venous thromboembolism in patients with active cancer is common and great variability in incidence rates is seen with different cancer types. Those cancer types with high incidence of VTE should be considered thromboprophylaxis research.

HEM06

Fibrin structure assay: a new diagnostic tool in the management of cancer

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Background: VTE is a common cause of death associated with cancer. Cancer patients with chronic inflammation have been reported to have an abnormal fibrin structure. The nanostructure concept previously described [1] has been proposed for determining the thrombotic risk in hypercoagulation states such as cancer.
Aims: Identify cancer patients with abnormal fibrin structure in association with cancer stage, inflammation and clot-lytic balance.
Methods: A collection of 34 cancer patients has been documented with type of cancer, tumoral stages and Khorana index for VTE risk. Cancer patient plasmas were compared with those from 84 healthy donors using fibrin structure assay [1], PAI activity assay and fibrinogen. Fibrin structure parameters (number of protofibrils Np) were plotted against temporal ones (fibrin formation and lysis time, or clot-lysis ratio) to classify plasmas into different groups according to their profiles.
Results: Comparison on temporal parameters showed 32% of patient plasmas with normal clot-lysis profile, 60% with hypo-fibrinolytic profile, which goes in parallel to their high PAI activity levels, and 15% only with procoagulant tendency.
According to fibrin structure, 32% patients had both normal Np and clot-lysis ratio, 40% showed normal Np, but higher lytic resistance associated to high PAI values. These samples were mainly breast tumors at initial stages (1–2) and displayed intermediate risk of VTE according to Khorana′s index.
The 26% of patients with high Np associated to both high fibrinogen levels and normal clot-lytic balance had later cancer stages (3–4) and belonged to lung, stomach, colorectal and uterus tumors but were at low/intermediate Khorana′s VTE risk.
Finally, only 2 patients with both high Np and resistance to lysis were at high risk of VTE.
Conclusions: Fibrin structure assay is proposed as an interesting tool to help discriminating patients with abnormal clot structure, which could directly influence VTE risk and patient evolution. [1] Dassi et al. ISTH 15.

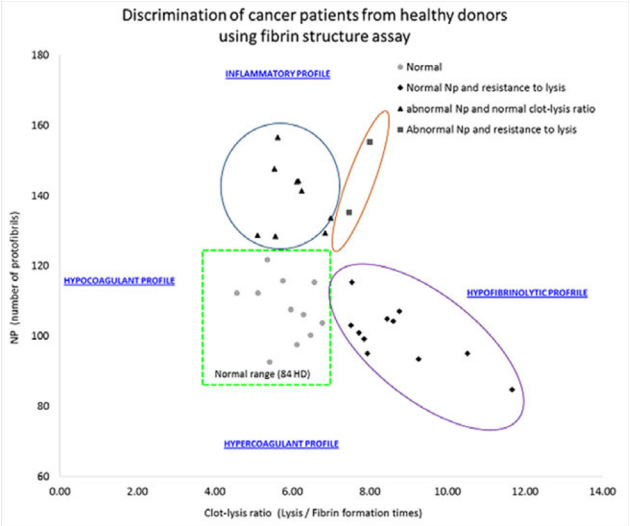

Figure Cancer patients in fibrin structure assay.

HEM07

Thromboembolic events (TE) in patients with metastatic non small cell lung cancer (NSCLC) treated with platinum-based chemotherapy

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Background: Venous thromboembolic disease is a common complication in cancer patients with a negative impact on their survival. In lung cancer, the scientific evidence for thrombosis during treatment with chemotherapy is limited.
Aims: The objective of this study was to describe the incidence of thromboembolic events (TE) in patients with metastatic NSCLC, occurring during treatment with platinum-based chemotherapy, in the Torrecárdenas Hospital.
Methods: It's about a retrospective study of 100 patients with stage IV NSCLC (EGFR/ALK native) receiving first-line platinum-based chemotherapy. TE happening since the beginning of chemotherapy to 1.5 months after the last administration, are described.
Results: Median age was 59 years (37–78). 80% male. Most frequent histology: adenocarcinoma (70%) and degree of differentiation G3 (57%). Visceral metastatic disease (60%). 70 patients were treated with cisplatin-based and 30 patients received carboplatin regimen. 13 patients (13%) experienced a TE during treatment (8 episodes of pulmonary thromboembolism and 5 episodes of deep vein thrombosis). 13% (9/70) of TE in patients exposed to cisplatin vs 10% (4/30) in patients exposed to carboplatin. Most events occurred in the first 3 cycles (60%). The median PFS was similar in patients with and without TE (7.5 vs. 8.1 months). The median OS was lower in patients with TE (7.1 vs. 11.7 months).
Conclusions: In our study, the incidence of TE in patients with metastatic NSCLC is higher than that described in previous studies. TE most occur in the first three cycles, and their appearance is associated with increased mortality. Adenocarcinoma, degree of differentiation 3, visceral metastases and chemotherapy based on platinum, are risk factors for TE in stage IV NSCLC.

HEM08

Cardiovascular effect of BCR-ABL TKIs: a meta-analysis and systematic review of arterial and venous occlusive events

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Background: A previous meta-analysis has demonstrated that dasatinib, nilotinib and ponatinib, 3 second or third generation tyrosine kinase inhibitors (TKIs) targeting BCR-ABL, are associated with increased risk of vascular occlusive events compared to imatinib in

patients with chronic myeloid leukaemia (CML). However, no distinction was provided concerning the arterial or the venous component of these events.
Aims: To assess and differentiate the risk of arterial and venous occlusive events in randomized controlled trials (RCT) of CML patients treated with these BCR-ABL TKIs compared with imatinib.
Methods: An update of our previous meta-analysis was performed by screening novel data published since October 21, 2014 to November 26, 2015. Two independent reviewers selected RCTs comparing a new generation TKI vs. imatinib in patients with CML, and extracted data for venous and arterial occlusive events according to a predefined classification sheet. The meta-analysis was performed using the random-effects model (REM) for the arterial subgroup analysis, whereas the fixed-effects model (FEM) was used for the venous analysis. Peto odds ratios (ORs) with 95%CI were computed.
Results: Twelve clinical trials were included. Ponatinib (REM OR_{PETO}: 3.26; 95%CI: 1.12–9.50), nilotinib (REM OR_{PETO}: 3.60; 95%CI: 2.21–5.86) and dasatinib (REM OR_{PETO}: 3.32; 95%CI: 1.37–8.01) are associated with an increased risk of arterial occlusive events compared with imatinib. Nonsignificant result has been found for bosutinib (REM OR_{PETO}: 2.77; 95%CI: 0.39–19.77). Overall, new generation TKIs increase the rate of venous occlusive events (FEM OR_{PETO}: 2.85; 95%CI: 1.04–7.78) but the stratification by treatment provided nonsignificant results.
Conclusions: New generation TKIs are associated with an increased risk of arterial and venous occlusive events. The analysis of individual level data including time-to-event will be needed to hypothesise potential mechanisms by which these events occurred.

HEM09

Effects of androgen deprivation therapy on hypercoagulability in prostate cancer patients - a prospective longitudinal study

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Background: Androgen deprivation therapy (ADT) is a common and effective therapy for advanced prostate cancer (PCa) but has significant adverse effects, including concern for cardiovascular and thrombotic complications.
Aims: To carefully examine the relationship between ADT use and hypercoagulability in PCa patients.
Methods: We performed a prospective longitudinal study in a cohort of PCa patients who had an indication for initiating ADT, including those with locally-advanced or metastatic disease (18 patients). Controls included men with lower-volume recurrent disease after curative therapy that were on watchful waiting not requiring ADT (10 patients) and also a cohort of healthy men with no prostate cancer (8 patients). Global hemostasis was evaluated using the sensitive global haemostasis assay thromboelastography (TEG). PCa patients were evaluated at baseline and every 3 months for at least 12 months.
Results: TEG demonstrated 14/18 (78%) of advanced PCa patients were already hypercoagulable before initiating therapy. Significant baseline hypercoagulability was documented in this cohort compared to those men on watchful waiting as well as the healthy controls. Interestingly, ADT did not appear to exacerbate the TEG findings over time. Overall ADT appeared to decrease hypercoagulability in this cohort of men as at the end of the study only 10/18 (56%) of PCa patients were hypercoagulable. However, 3/18 (16%) patients were found to have more hypercoagulable TEG readings on ADT compared to baseline.
Conclusions: This prospective study demonstrates a complex interaction between ADT and a hypercoagulable state in men with advanced prostate cancer. TEG abnormalities were mostly associated with stage or volume of cancer as compared to ADT use. However, it is possible that ADT may lead to hypercoagulability in only a subset of men suggesting that regular monitoring of coagulation of men on ADT could help identify those at risk of developing thromboembolic complications.

HEM11

Diagnostic performance of hemostatic biomarkers iin cancer patients

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Background: Accumulating evidence demonstrated a link between increased expression of hemostatic proteins and tumor progression. However, data on the diagnostic potential of serum hemostatic biomarkers in malignancy is still needed.
Aims: to analyze the diagnostic utility for detection of malignant state of microparticles expressing tissue factor (MPTF), tissue factor antigen (TF-Ag), angiopoietin-2 (ANG-2) and soluble urokinase plasminogen activator receptor (suPAR).
Methods: MPTF activity, plasma TFAg, suPAR and serum ANG-2 were determined in 128 cancer patients and 82 matched healthy controls. MPTF activity was measured with Zymuphen MPTF kit; TFAg, suPAR and ANG-2 were quantitated by ELISA. Study was approved by the ethics committee at Medical University - Plovdiv and written informed consent was obtained from all participants. To analyze the diagnostic utility of the hemostatic biomarkers receiver operating characteristic (ROC) analysis was performed. Sensitivity, specificity, positive and negative predictive values were determined and area under the curve (AUC) constructed for each parameter.
Results: MPTF activity was significantly lower in patients than in controls, whereas TFAg, suPAR and ANG-2 were significantly elevated, $P < 0.0005$. ROC analysis showed that MPTF at an optimal cut off ≤ 2.8 pg/ml had sensitivity of 96.87% and specificity of 59.76%, AUC of 0.715 ($P < 0.0001$, CI 0.649–0.775). TFAg (cut off >207.1 pg/ml) exhibited sensitivity 47.12% and specificity 85%, AUC 0.631 ($P < 0.0016$, CI 0.557–0.701). ANG-2 (cut off >266.3 pg/mL) had sensitivity 69.23% and specificity 76.6%, AUC 0.762 ($P < 0.0001$, CI 0.681–0.831). suPAR at cut off >9.1 ng/ml had sensitivity 34%, specificity 100%, AUC 0.696 ($P < 0.0001$, CI 0.609–0.774). ROC curve comparison revealed significantly better diagnostic performance of MPTF, ANG-2 and suPAR than TFAg. See Figure.

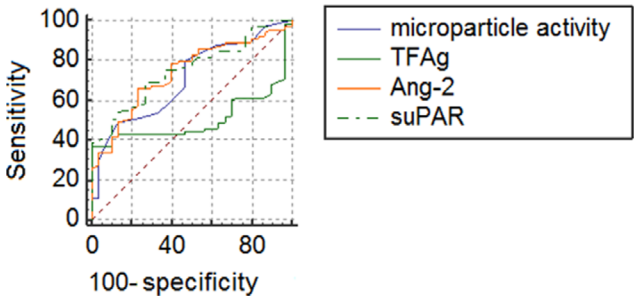

Figure ROC curves comparison.

Conclusions: MPTF, ANG-2 and suPAR exhibit reliable discriminating ability and could serve as predictors for the presence of malignancy.

HEM12

Evaluation of thromboelastography (TEG) in patients with myeloproliferative neoplasm (MPN): platelet count > 600 associated with prothrombotic parameters

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Background: MPN is an independent risk factors for cardiovascular and thrombotic events. However, there currently remain no routinely available laboratory tests to evaluate these risks. Thromboelastography (TEG) may be a better surrogate measure of these individual's thrombosis risk.
Aims: Determine correlation between TEG and MPN.
Methods: MPN patients regardless of treatment status were recruited. All samples were citrated and underwent TEG 5000S analysis within 4 h of collection. Routine TEG parameters including R (mins), K (mins), maximum amplitude (MA), α-angle and lysis percentage were recorded. Results were compared to normal controls.
Results: 29 MPN patients (15 F, 14 M) with median age of 67 (45–82) were recruited. 21 had essential thrombocytosis (ET) and 7 with polycythaemia rubra vera (PRV); 24 were JAK2 positive, 4 calreticulin (CALR) positive and 1 was JAK2 and CALR negative. No differences were seen between ET and PRV. When compared to age-matched normal controls, MPN patients with platelet count >600 had more thrombotic parameters (see table). Interestingly, and contrary to other prothrombotic markers, lysis (LY30), a marker of clot breakdown, was significantly higher (3.05% vs 0.3%, $P < 0.001$). These changes were independent of aspirin use. Similarly, patients with at least 2 TEG parameters within the top quartile of age-matched normal control range, had higher platelet counts (588 vs 429, $P = 0.01$), thrombin generation (760 vs 687, $P = 0.03$), higher lymphocyte count (2.1 vs 1.4, $P = 0.01$) and more likely to be on cytoreductive therapy (68% vs 27%, $P = 0.03$).
Conclusions: This pilot study suggests that MPN patients with platelets >600 have more prothrombotic TEG parameters. Similarly, the presence of 2 or more "prothrombotic" TEG parameters, when compared to age-matched controls, predicts for thrombocytosis, use of cytoreductive therapy and higher thrombin generation.

HEM13

Cancer, Chronic Kidney Disease and Thrombosis

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Background: C-KIN (Cancer & the Kidney International Network) was created in 2014 and aims at improving the management of cancer patients by developing clinical and scientific knowledge on the treatment of cancer in chronic kidney disease (CKD) patients. A specific working group: Thrombosis, Kidney disease, and Cancer (TKC) has been created.
Aims: The aim was to present the summary of the scientific rationale on the links between cancer, CKD and VTE (venous thromboembolism).
Methods: C-KIN TKC working group reviewed the literature (PubMed) and investigated about the potential links between.
1) cancer-VTE,
2) CKD-VTE and
3) cancer-CKD.
Results: VTE is a risk factor of cancer. The incidence rate of cancer during the 1st year after VTE is 60.6/1000 patient-year (vs 9.5 in patients without VTE). Furthermore, a VTE event is associated with a 4.0-fold higher risk of cancer during the 1st year after VTE and a 1.3-fold (95% CI 1.12–1.53) higher risk for subsequent years.
CKD is frequent (18.1–36.7%) and it is increasing in VTE patients. Furthermore, VTE patients with CKD are at an increased risk for recurrent VTE and major bleeding. Finally, CKD is an increased risk of mortality in VTE patients.
CKD patients are at higher risk to develop cancer. This risk begins for a glomerular filtration rate (GFR) < 55 ml/min/1.73 m² and increases linearly as GFR declines. On the other hand, CKD is frequent (12–25%) in cancer patients and CKD is a risk factor of mortality in cancer patients. Finally, every 10 ml/min/1.73 m²-decrease in GFR is associated with an 18%-increase in cancer related mortality.
Conclusions: These 3 diseases are closely linked. It is important to screen and manage both cancer and CKD diseases in VTE patients and to reduce the dose of all medications in these patients if necessary. However, the trends and risks in VTE patients presenting both comorbidities (cancer and CKD) and their potential cumulative effects have not yet been clearly evaluated and there is a need of guidelines in this specific situation.

HEM14

Thromboprophylaxis for prevention of venous thromboembolism in patients with multiple myeloma - a systematic review and metaanalysis

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Background: Incidence of venous thromboembolisc events (VTE) in patients with multiple myeloma during treatment with immunomodulatory chemotherapy regimens is high. The safety and efficacy of thromboprophylactic agents in this patient population for VTE prevention is uncertain.
Aims: Assess the safety and efficacy of various pharmacological thromboprophylactic agents for VTE in patients with multiple myeloma who receive chemotherapy.
Methods: Databases searched include MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews (CDSR), Database of Abstracts of Reviews of Effectiveness (DARE) and ISI the Web of Science, World Health Organization (WHO) International Clinical Trials Registry Platform and Current Controlled Trials. Annual conferences of International hematology and oncology Societies were hand searched for abstracts.Randomized trials comparing pharmacological thromboprophylaxis in patients with multiple myeloma treated with chemotherapy were included. Quasi randomized or non randomized studies were excluded. Comparisons of thromboprophylaxis with agents such as aspirin,unfractionated heparin, LMWH, fondaparinux, warfarin, other vitamin k antagonists or new oral anticoagulants were performed**.** Two authors assessed trials for inclusion and extracted data independently and RevMan was used for data analyses.
Results: 2 RCT out of 970 citations were included in the meta analysis. Overall methodology of the studies was moderate. Meta analysis showed no differences in the composite outcome, symptomatic DVT,

PE, major or minor bleeding between Aspirin and LMWH. There was significant reduction in the grade 3–4 VTE with LMWH compared to warfarin (RR 0.33, 95% CI 0.14 TO 0.83) based on a single study.
Conclusions: Choice of thromboprophylactic agent for VTE prevention in patients with Multiple Myeloma treated with immunomodulatory therapy remains unclear. There were no significant differences between the three regimens in terms of outcomes.

HEM15

Risk assessment of venous thromboembolism (VTE) and thromboprophylaxis in pregnant women hospitalized with cancer

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Background: Patients with cancer are considered high risk for developing venous thromboembolism (VTE) when hospitalized.This risk is even greater when associated with pregnancy, which itself increases the risk of thrombosis. There is no data in the literature that assess THE risk of VTE in women with cancer in pregnancy and hospitalization.
Aims: Evaluate the application of a thromboprophylaxis protocol with VTE risk score for pregnant women hospitalized with diagnosis of cancer to clinical and/or surgical treatment. Assess the impact of application of this protocol in the prevention of maternal morbidity and mortality from VTE.
Methods: Longitudinal and prospective study of pregnant women with cancer diagnoses admitted in HCFMUSP in the period of Dec 2013 to Aug 2015. Application of a thromboprophylaxis protocol with VTE risk score (see Table). The collected data were analyzed descriptively, identifying the profile of pregnant women, type of cancer, using percentages, absolute values.
Results: We evaluated 61 cases. 12 cases were classified as low risk (score < 3) and 80% scored high risk. Of the 49 remaining cases, two were already in anticoagulation (previous deep vein trombosis and lymphoma). Of the 47 cases of high risk (score ≥ 3), 41 received enoxaparin in prophylactic dose and six cases had contraindications to prophylactic medication. Chemotherapy in the last 6 months: 32 cases, age ≥ 40 years: 10 cases, age ≥ 35 and < 40: 13 cases, multiparity: 11 cases, infection: 5 cases; BMI> 40- 1 case. Type of cancer among 47 high risk cases: Cervical cancer: 4 (8.51%); breast cancer: 33 (70.21%), lymphoma:3 (6.38%), leukemia: 4 (8.51%), others: 3(6.38%). No patients developed VTE, adverse effects of anticoagulation or death until 3 months post hospitalization.

Table VTE risk score for hospitalized pregnant women.

SCORE 3	SCORE 2	SCORE 1
previous thrombosis/ thromboembolism	Protein C deficiency/Protein S deficiency	Age ≥ 35 and ≤39 y
Homozygous mutations	Heterozygous FV Leiden/ heterozygous FII G20210A mutation	Parity ≥3
Combined thrombophilia risk factors	Cancer(last 6 months)	Multiple pregnancy
Antiphospholipid syndrome	Chemotherapy(last 6 m)	Hyperemesis
Cancer(stomach, pancreas, lung)	Current serious infections	Gross varicose veins
Inflammatory acute conditions (autoimune diseases)	BMI≥40 kg/m2	Smoker ≥20
Sickle cell disease	Age≥40y	Surgical procedure(except C-section)
Nephrotic syndrome	Lung disease(cyanosis)/ postpartum hemorrhage >1L	
Heart disease	Immobility,bed rest >4d prior to C-section	

Conclusions: The majority of pregnant women with cancer had a high risk for TEV. Breast Cancer was the most prevalent. Chemotherapy in the last 6 months was the main factor that scored high.

HEM16

Tissue factor positive microparticles remain undetectable by flow cytometry in pleural fluids from cancer patients despite high level of procoagulant activity

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Background: The feasibility of detecting tissue factor (TF) expressing microparticles (MPs) by flow cytometry (FCM) is a matter of debate since this approach is prone to artifacts and discrepancies between MP-associated TF-specific procoagulant activity (MP-TF PCA) and FCM-based data have been described. Pleural fluids from cancer patients can harbour EpCAM+ MPs and this has been proposed as a diagnostic marker for malignant effusions (1). With tumor-derived MPs in these fluids, both a high MP-TF PCA and TF+ MPs (MP-TF) may be expected.
Aims: Both approaches were applied to n = 12 samples to compare data.
Methods: Malignant pleural fluid samples were collected as described (1). MP-TF PCA was operated on total MPs extracted from frozen aliquots of pleural fluids using a FXa generation assay modified from Lee, JTH 2011 with sensitivity limit below 10 fM TF (2). FCM detection of MP-TF was standardized on a 3-laser Gallios cytometer with cutoff set at 0.3 μm-eq in FSC (3). Specific care actions (4) included i) multi-color discrimination of MP subsets, ii) addition of hirudin to avoid micro-coagulation, iii) reagents pre-clearing, iv) negative controls with detergent-based MP lysis.
Results: On 12 fluids with detailed immuno-phenotype, MP-TF remained un-detected despite i) high levels of MP-TF PCA, most often >100 fold higher than normal values in plasma (>1.5 pM TF vs ~15 fM), ii) presence of EpCAM+, likely tumor-derived, MPs.
Conclusions: Bio-assays are more appropriate than immuno-assays to detect MP-TF in pleural fluids. Immunological detection of TF on MPs remains a challenge even though major care is applied in FCM protocols and explosive levels of MP-TF PCA are measured. Whether MP-TF PCA may complement the immunological detection of EpCAM+ MPs for mini-invasive identification of patients with malignant pleural effusions remains to be established. Refs: 1) Roca, Oncotarget 2015, 2) Agouti, BJH 2015, 3) Poncelet, Cytometry A 2015, 4) Poncelet, TRASCI 2015.

HEM17

Microparticle-tissue factor activity as biomarker for excluding thrombosis in acute leukemic patients: comparison of three assays

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Background: Thrombosis induced by a hypercoagulable state is a common complication in patients with malignancies. Leukemic cells shed

procoagulant microparticles bearing tissue factor (MPs-TF) which may play a major role in venous thromboembolism (VTE) or disseminated intravascular coagulation (DIC) in acute leukemia (AL).
Aims: To compare the performance of 3 MP-TF dependent bio-assays to exclude a thrombotic risk in AL patients.
Methods: Blood samples from 44 patients with newly diagnosed AL were obtained at Day0 (before treatment), D3 and D7 (after treatment). Procoagulant activity (MP-PCA) was assayed by i) thrombin generation (TG) on MPs purified by ultra-centrifugation and spiked in normal plasma and by two TF-specific FXa generation assays run in synthetic medium, ii) first in-house assay run on total MPs pelleted by high-speed centrifugation and iii) second assay run on immuno-captured MP-TF (Zymuphen MP-TF®). Cutoffs for exclusion of thrombosis were calculated for each test using ROC curves.
Results: Among all 44 AL patients, 5 had an increased MP-PCA in TG and 4 of them developed a thrombotic event. All patients without thrombotic event except one showed MP-PCA under cutoff value. By FXa generation assay on centrifuged MPs, MP-TF PCA showed results similar with the TG assay. All patients without thrombotic complication had MP-TF activity values under the cutoff (85 fM TF). Patients with DIC or VTE clearly showed higher values. By the assay on immuno-captured MP-TF only 4 patients had an increased activity (>2 pg/ml), among whom 3 developed a thrombotic event and one had haemorrhage. Surprinsingly, one patient with DIC had a normal activity level. Elevated MP-PCA was quickly reduced by treatment.
Conclusions: In the present small series of patients with AL, TF-focused procoagulant bioassays operated on purified MPs were useful to rule out a high risk of thrombosis, with negative predictive value from 97.5% to 100% depending on the assay.

HEM18

Short- and long-term mortality after pulmonary embolism in patients with and without cancer

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Background: Pulmonary embolism (PE) is a major cause of mortality and morbidity. It is known that the risk of death varies by provoking factors, however, it is unknown if the risk of death persists beyond the initial diagnosis among patients with cancer associated non-cancer provoked patients.
Aims: In this study, we aimed to investigate the effect of cancer on overall, short- and long-term mortality in a cohort of consecutive incident PE patients.
Methods: Using the administrative health care databases of the Canadian province of Alberta, we identified all incident cases of pulmonary embolism between 2004 and 2012 and stratified them by provoking factors (unprovoked, provoked, and cancer-associated). Multivariate Cox survival model was used to estimate the hazard ratios of short- and long-term death.
Results: We identified 8641 patients with PE, among which 42.2% were unprovoked, 37.9% were provoked and 19.9% were provoked by cancer. The 1-year and 5-year survival probabilities were 61% (95% CI: 57%-0.64%) and 39% (95% CI: 36–43) in cancer-associated PE patients, 93% (95% CI: 92–94) and 80% (95% CI: 78–81) in provoked PE patients, and 94% (95% CI: 93–95) and 85% (95% CI: 83–87) in unprovoked PE patients, respectively. Compared to patients with unprovoked events both short-term and long-term survival in patients with cancer associated PE have significantly higher observed risk of all-cause mortality in all age groups, P-value < 0.001. In contrast, patients with provoked events had similar short- and long-term hazard of death.
Conclusions: PE is still a common condition with a high mortality in all risk groups, however, patients with cancer have a substantial risk of short-term mortality compared to patients with unprovoked PE.

HEM19

Impedance platelet aggregometry for surveillance of ibrutinib therapy in chronic lymphocytic leukaemia

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Background: Treatment with ibrutinib, an orally administered inhibitor of Bruton's tyrosine kinase (BTK), has revolutionised therapy of Chronic Lymphocytic Leukaemia (CLL), especially in patients with relapsed/refractory disease, with an overall survival rate of 83% in a recently published 3-year follow-up. Bleeding is a common adverse event in up to 61% of patients, usually grade 1 AEs. Bleeding tendency results from BTK-expression in platelets as BTK has been found to be involved in GPVI (collagen) as well as GPIb (von Willebrand factor) induced signalling.
Aims: Platelet function using impedance platelet aggregometry might be helpful for prediction of bleeding risk.
Methods: 64 ibrutinib-treated CLL patients (median age 71 years, 64.1%male) from 4 centres (Vienna, Salzburg, Munich and Cambridge) were seen for regular clinical examinations and platelet function testing. 287 measurements (median 3 measurements per patient, range: 1 - 14) were available for analysis. Concomitant antiplatelet and/or anticoagulant medication was present in 28.1%. Median observation time was 11 months (range: 0.5 - 35.3) and bleeding occurred in 39/64 (60.9%) patients. Impedance aggregometry was performed using full-blood samples in a Roche Multiplate® Analyser.
Results: Ristocetin and collagen induced platelet aggregation were significantly impaired when bleeding occurred ($P < 0.0001$ and $P = 0.0015$ respectively). Platelet count was lower in bleeding compared to non-bleeding patients ($P = 0.005$). Impairment of platelet aggregation was still present after patients were grouped by platelet count >100x10*9/l or < 100 × 10*9/l ($P < 0.0001$ and $P = 0.003$ respectively). 87 bleeding events were observed during follow-up (61 grade 1, 24 grade 2, 2 grade 3 and no grade 4/5 events). No severe bleeding event was observed at a ristocetin induced platelet aggregation value > 36 [U].
Conclusions: Monitoring platelet function could provide evidence for clinical decisions such as dose reduction, anti-coagulation and planning surgery. Further research is warranted.

HEM20

Platelet transfusion practices in adult surgical, haematology and oncology patients

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Background: Platelet transfusions are indicated for the prevention and treatment of hemorrhage in patients with thrombocytopenia or platelet function defects. Platelet transfusion should be judicious and must be given only when there is clear clinical justification in order to reduce risks associated with transfusion, costs from production and possible shortages.
Aims: To evaluate current practice of platelet transfusions in adult surgical, haematology and oncology patients against standards drawn from the BCSH (British Committee for Standardization in Hematology) guidelines for platelet transfusions.
Methods: A retrospective analysis of platelet transfusion therapy given to adult surgical, hematology and oncology patients was done in the

month of June 2015. Medical charts of all patients who received platelet transfusion during study period were reviewed. The record was evaluated to review primary diagnosis, platelet count and indications for platelet transfusion. Current practice of platelet transfusion were compared against standards drawn from the BCSH.
Results: Total number of platelet transfusions was 141 in month of June 2015. Number of episodes(n) of platelet transfusion in hematology was n = 101/141 (72%), in surgery n = 26/141(18%) and in oncology n = 14/141(10%).

Table Indications of Platelet Transfusion.

DEPARTMENT	PROPHLACTIC TRANSFUSIONS	THERAPEUTIC TRANSFUSIONS
Haematology	90% (*n* = 91/101)	10% (*n* = 10/101)
Oncology	71% (*n* = 10/14)	29% (*n* = (4/14)
Surgery	27% (*n* = 7/26)	73% (*n* = 19/26)

Table Justified Platelet transfusions.

DEPARTMENT	JUSTIFIED (*n* = number of platelet transfusions)	Not Justified
Haematology	*n* = 90/101 (89%)	*n* = 11/101(11%)
Oncology	*n* = 10/14 (71%)	*n* = 4/14 (29%)
Surgery	*n* = 8/26 (31%)	*n* = 18/26 (69%)

Conclusions: The study demonstrated that 77% of platelet transfusions were done for prophylactic purpose. There was a high rate of adherence of platelet transfusion to BCSH standards in haematology department followed by oncology. However the policy was significantly breached in surgical where platelets were mostly transfused for therapeutic reasons i.e. active bleeding due to surgically correctable cause.This indicates an area for intervention in the form of physician's education to rationalize the use of platelet product in surgical department.

HEM21

Platelet-derived factor v is an important determinant of the metastatic potential of circulating tumor cells

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Background: Factor V (fV) is an essential component in the blood coagulation cascade. FV inhibitors in patients have been associated with malignancy. fV is distributed in plasma and platelet pools distinguished by physical and functional differences. The roles of fV in malignancy remain understudied.
Aims: To directly determine whether factor V is determinant of the metastatic potential of circulating tumor cells, this study examined the impact of different level of fV gene expression restricted to either the plasma or platelets on the hematogenous pulmonary metastasis of established murine tumors.
Methods: In the current study, we report that platelet fV is critical for the regulation of metastasis in murine B16-F10 metastasis models *in vivo*, with adhesion and transendothelial migration assays *in vitro*.
Results: In murine B16-F10 metastasis models, the transgenic mice with lower platelet fV exhibited marked reductions in metastases compared with mice with higher platelet fV. *In vitro*, B16-F10 melanoma cells showed increased adhesion to and transmigration through endothelium treated with lower level of platelet fV, but not plat platelet poor plasma.
Conclusions: These findings suggest that platelet-derived fV contributes to the control of tumor metastasis, and is likely associated with regulation of tumor cell adhesion and transmigration.

HEM22

The hypercoagulable profile of patients with myeloproliferative neoplasms (MPN) as detected by whole blood rotational thromboelastometry (ROTEM)

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Background: Essential Thrombocythemia (ET) and Policythemia Vera (PV) are clonal disorders of hematopoietic stem cells characterized by hypercoagulability and an increased incidence of thrombosis. ROTEM, which is a global coagulation assay, can provide a complete hemostatic profile of MPN patients.
Aims: This study aims to assess the feasibility of using thromboelastometry to characterize the prothrombotic state of MPN patients and to evaluate its correlation with mutational status and treatment.
Methods: Citrated blood was collected from 39 ET, 23 PV patients and 19 healthy subjects upon informed consent. Analysis was performed to evaluate the intrinsic (INTEM) and extrinsic (EXTEM) pathway. Maximum clot firmness (MCF [mm]), which reflects the maximum tensile strength of the thrombus, and clotting formation time (CFT [sec]), namely the time that clot takes to increase from 2 mm to 20 mm above baseline, were recorded.
Results: ROTEM analysis showed a hypercoagulable profile in MPN patients, who had shorter CFT and higher MCF compared to controls. In ET and PV patients, a high statistically significant ($P < 0.01$) correlation was found between platelet count and MCF or CFT. Multivariate analysis showed that only platelet count was independently associated to ROTEM results. To correct for platelet differences, a ratio between MCF and the respective platelet value (rMCF) was created. Interestingly, rMCF was significantly lower in patients compared to controls ($P < 0.01$), in ET compared to PV ($P < 0.05$) and in calreticulin-positive subjects ($P < 0.05$), while was higher in patients under cytoreductive therapy (P =ns).
Conclusions: This study confirms the occurrence of a hypercoagulable state in ET and PV patients; however, when MCF values were corrected for platelet count, the platelet activity was lower in MPN patients than controls, supporting the hypothesis that platelet function is exhausted upon clotting activation.
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HEM23

Thrombosis in myeloproliferative neoplasia associated with different coagulation factor polymorphisms

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Background: The group of myeloproliferative neoplasms (MPNs) are known for their different phenotypes but similar complications: vascular events. Blood cells, interaction between them, and the activation of coagulation factors play a role in the pathogenesis of thrombosis in MPNs. Platelet-specific polymorphisms, FVII as well as β-fibrinogen single nucleotide polymorphisms (SNP's) have been never analyzed together in patients with MPNs.
Aims: To evaluate the effects of coagulation factor VII, β-fibrinogen and TT genotype of GP c.807C>T single nucleotide polymorphisms, and the risk of thrombosis in patients with PV, ET, and PMF at the Department of Oncology and Hematology of the Institute of Oncology, the Lithuanian University of Health Sciences.
Methods: We included 108 patients in this survey. Findings of clinical and hematological analyses were collected. Genotyping was done using PCR and PCR-RFLP analysis.
Results: TT genotype of GP c.807C>T polymorphism was more frequently found in the group of MPN patients with arterial thrombosis compared to the MPN patients who were thrombosis-free (26.5% vs. 11.5%, $P = 0.049$). CT genotype of β-fibrinogen c.-148C>T polymorphism occurred significantly more frequently in MPN patients with arterial and total thrombosis compared to the wild type or homozygous genotype (respectively, 57.7% vs. 40.0 vs. 12.5%; $P = 0.027$), (64.7% vs. 44.4% vs. 25% $P = 0.032$). The carrier state for 323P10 variant of FVII SNP (summation of P10/10 and P0/10) was significantly more frequent in MPN patients with total thrombosis compared to the wild-type genotype carriers (71.4% vs. 43.4%, $P = 0.049$). The coexistence of both genotypes - heterozygous β-fibrinogen c.-148C>T and FVII -323P0/10 SNP - statistically significantly increased the odds of arterial thrombosis in MNP patients (21.1% vs. 3.7%, $P = 0.008$).
Conclusions: We found that 323P10 variant of FVII, TT genotype of GP c.807C>T and CT genotype of β-fibrinogen c.-148C>T SNP polymorphism may be associated with risk of thrombosis in patients with MPNs.

HEM24

Evaluation of mean platelet volume as a predictive marker for venous thromboembolism in patients treated for hodgkin lymphoma

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Background: It has been suggested that mean platelet volume (MPV) is associated with the risk of venous thromboembolism (VTE) in patients with cancer.
Aims: We evaluated the association of MPV with VTE in patients treated for Hodgkin Lymphoma (HL).
Methods: Retrospective analyses for symptomatic VTE were performed on 167 adult patients (median age 37, range 18–79 years, of whom 54% were females) with HL.
Results: Analysis of the risk of thrombosis using the Khorana VTE risk assessment model showed an intermediate risk in 132 patients (1–2 points) and high risk in 35 patients (3 or more points). During the observation period (median 32 months), 12 (7.18%) patients developed VTE during first-line treatment. VTE occurred in 2 patients of the high-risk group (17%) and in 10 patients (83%) of the intermediate group of the VTE-risk scoring model. The pre-chemotherapy values of MPV were significantly lower in patients who developed VTE in comparison to patients without VTE ($P = 0.0342$). Patients with MPV≤25th percentile (6.8 fl) had an increased risk of developing VTE.
In univariate analysis, MPV≤25thpercentile (OR 2.16; 95%CI 1.12–4.17, $P = 0.0215$) and advanced stage IV (OR 2.28; 95%CI 1.22–4.27, $P = 0.0097$) were associated with the occurrence of VTE. Other patient-related factors; age, gender, disease-related factors; International Prognostic Score, presence of constitutional symptoms and VTE risk assessment model score according to Khorana model, failed to be prognostic for VTE.
In multivariate analysis, MPV≤25thpercentile (OR 2.36; 95%CI 1.24–4.49, $P = 0.009$) and advanced stage (stage IV vs. stage I-III, OR 2.28; 95%CI 1.13–4.57, $P = 0.027$) remained significant factors for developing VTE.
In a Kaplan-Meier analysis, patients treated for HL without VTE had a higher probability of survival than patients who developed VTE.
Conclusions: Our results suggest that the pre-chemotherapy MPV value is a readily available parameter that may be a useful prognostic marker for a significant risk of VTE in patients with HL.

HEM25

Risk factors assessment for thrombosis in patients with cancer - research project of the federal university of minas gerais

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Background: Venous Thromboembolism is a possible and frequent complication in patients with cancer, representing an important cause of morbidity and mortality, moreover, particularly idiopathic events may be considered as an epiphenomenon of a hidden tumor. VTE is the main direct cause of death in patients with cancer, and pulmonary embolism (PE) three times more frequent in these compared to patients without cancer.
Aims: To evaluate the clinical and laboratory risk factors for venous thromboembolism in patients with cancer. We evaluate the predictive value for VTE of D-dimer testing, factor VIII, microparticles, thromboelastometry and thrombin generation test at the pre-treatment period, as well as the importance of family history, age and other acquired factors.
Methods: Patients to be included in the study will have solid tumors, first diagnostics not yet subjected to any treatment, that are admitted to the hospital out-patients unit. From these patients will be collected demographic data in a standardized questionnaire as well as tests to research for the most common thrombophilic disorders, and Khorana Risk Factors will be calculated. All patients will be instructed to seek emergency care if there is clinical suspicion of thrombosis, if confirmed wig image tests, anticoagulantion will be initiated, in the acute setting with low molecular weight heparin (LMWH) or rivaroxaban and followed with LMWH or antagonists of vitamin K. This patients will be monitored during 6 months for recurrence of thrombosis and bleeding.
Results: This project is currently under initial steps. Assuming that the incidence of VTE in low-risk population is approximately 0.8% and the high risk of 7.1% (according to Khorana score), an alpha error of 0.05 and a power of 80%, the minimum sample size in each group is 147 patients.
Conclusions: This project will try to validate the Khorona Score in our Institution.

HEM27

Frequency and management of venous thrombosis in adult acute leukemia patients at a tertiary care hospital of Pakistan

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Background: The incidence of thrombosis is not widely studied in patients with acute leukemia and their management is a great challenge. This may be obscured by significant morbidity and mortality due to complications such as bleeding and infections. No established guidelines are present to treat these difficult patients. Case-controlled studies of patients with cancer revealed a fourfold increase in thromboembolic occurrence in acute leukemia, with about the same rate in acute myelogenous leukemia and in acute lymphocytic leukemia. Among patients with acute leukemia, thrombosis has the highest incidence in acute promyelocytic leukemia.
Aims: To determine the frequency of venous thrombosis and treatment strategy in patients with acute leukemia at a tertiary care Hospital of Pakistan.
Methods: Retrospective, observational study of case charts of hospitalized patients with diagnosed case of acute leukemia at department of oncology Aga Khan University Hospital Karachi during the 18 months period (January 2014 to June 2015). Data was retrieved by using ICD 9 coding for acute leukemia patients. Investigations were obtained from electronic medical record system. Finally data was analyzed for frequencies and percentages by using SPSS version 19.
Results: Total of 107 patients presented during the study period. Among them 76 were males and 31 were females with median age ranges from 18 to 60 years. These patients were stratified into 2 major groups according to type of leukemia.63.5% patients were with Acute myeloid leukemia in which 4.7% patient developed venous thrombosis among them highest in APML 22.2% while 36.4% patients were with acute lymphocytic leukemia in which 2.5% of patients developed venous thrombosis. Three patients were treated successfully with LMWH during their consolidation phase of chemotherapy.
Conclusions: Venous thrombosis in acute leukemia is not uncommon which can lead to fatal results. Anticoagulation with intermittent use of LMWH for 3–6 months would be the appropriate option for treatment.

HEM29

Contribution of modelling of procoagulant properties of cancer cells in the understanding of their mechanisms of action and the effectiveness of anticoagulant agents

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Background: The pathogenesis of the prothrombotic state in cancer is complex and may alter the efficiency of the antithrombotic agents but remains unclear.
Aims: Dissection of the mechanisms responsible for the procoagulant activity of pancreas adenocarcinoma (BXPC3) and human breast carcinoma cells (MCF7) by thrombin generation assay (TG) in different relevant conditions and the study of their influence on the antithrombotic efficiency of apixaban, fondaparinux and enoxaparin.
Methods: Cells were cultured in 96-well plates and normal platelet poor or rich plasma (PPP; PRP) spiked or not with apixaban, fondaparinux or enoxaparin was added. TG was done with CAT® assay in different conditions of reagents, with anti-tissue factor antibody (anti-TF), or corn trypsin inhibitor (CTI). Alternatively spliced TF (asTF), TF activity (TFa) and cancer procoagulant (CP) were also assessed.
Results: The TFa and asTF were found in abundant amounts in BXCP3 than MCF7 cells. The CP levels were higher in MCF7. BXPC3 amplified TG more than MCF7. Anti-TF inhibited TG triggered by BXCP3 and MCF7. The CTI had pronounced inhibitory effect on TG triggered by MCF7. TG enhancement by BXPC3 and MCF7 was mediated by FVII. Factor XII was more important for TG enhancement by MCF7. Comparison on the basis of IC50 showed that in the presence of BXPC3 or MCF7 the efficiency of apixaban was preserved. Fondaparinux, was more vulnerable to the presence of cancer cells as compared to apixaban. The effect of BXCP3 or MCF7 cells on the antithrombotic potency of enoxaparin was of similar magnitude as that on apixaban.
Conclusions: Mechanism coagulation activation by BXPC3 is dominated by TF pathway. MCF7 additionally imply FXII activation. The type of cancer cells is determinant for the antithrombotic efficiency of the antithrombotic agents. Modeling procoagulant profile of cancer cells provides an understanding of the procoagulant mechanisms and could evaluate the efficiency of antithrombotic treatment.

Lupus Anticoagulant/ Antiphospholipid Antibodies

LUP01

Inverted erythrocyte membranes as a novel model for studying the antiphospholipid syndrome

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Background: The antiphospholipid syndrome (APS) is an acquired autoimmune disorder predisposing patients for thrombosis or pregnancy complications. Diagnostic testing is based on detection of APS autoantibodies targeting anionic phospholipids (PL) binding β_2-glycoprotein I or detection of the lupus anticoagulant effect.
Aims: The aim was to study the interaction of APS antibodies and PL-binding proteins with inverted erythrocyte membranes (iEMs) as a source of naturally occurring anionic PL.
Methods: Plasma samples were obtained from consenting patients with documented APS and healthy controls. iEMs were prepared by hypotonic lysis of erythrocytes. Phosphatidylserine (PS) exposure was detected by annexin A5 staining. IgG binding to iEMs was investigated using goat anti-human IgG by flow cytometry and confirmed by gel electrophoresis and western blot. Thrombin generation (TG) was performed with iEM at 5 pM tissue factor.
Results: Over 95% of iEMs exposed PS as detected by annexin A5 staining. APS patient plasmas demonstrated higher levels of IgG binding to iEMs as compared to healthy controls (median fluorescence intensity [IQR]: 1362 [1053–2284] (n = 10) vs. 654.9 [611.6–800.7] (n = 9); $P = 0.0002$) (Figure 1A). The higher levels of IgG binding were confirmed by gel electrophoresis, with a significant difference for IgG heavy chain density (mean ± SEM, APS: 1.42 ± 0.44 (n = 4) vs. normal: 0.30 ± 0.03 (n = 4); $P = 0.0286$) (Figure 1B).

TG increased with increasing concentrations of iEMs, indicating that iEMs provide adequate anionic PL for coagulation proteins to bind (Figure 2A). The lupus anticoagulant effect was observed in TG when comparing APS patients (n = 5) with healthy donors (n = 5) (median lag time [IQR]: 6.0 [5.15–7.85] vs. 2.0 [1.75–2.25]; P = 0.0079) (Figure 2B).

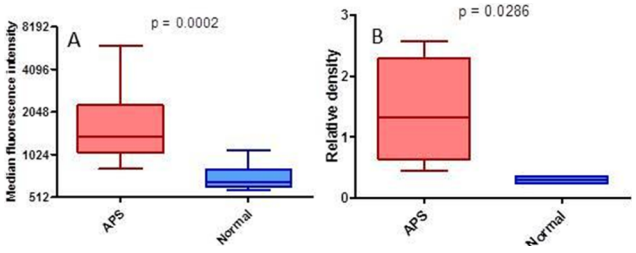

Figure 1

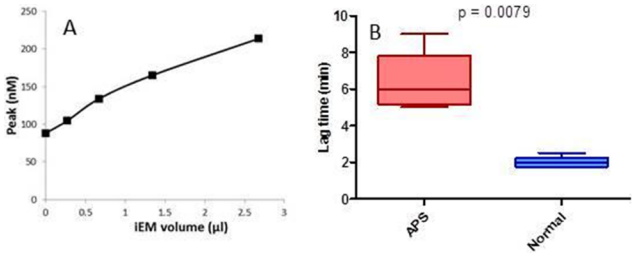

Figure 2

Conclusions: iEMs expose anionic PL necessary for binding of proteins to which APS antibodies are directed. Therefore, they are a promising, more physiological model to study the interaction with APS antibodies and coagulation factors.

LUP02

Pravastatin ameliorates preeclampsia and/or severe IUGR in 13 women with refractory obstetric antiphospholipid syndrome (APS)

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Background: Despite the administration of conventional treatment for obstetric APS (OAPS) (e.g. low dose aspirin (LDA) plus low molecular weight heparin (LMWH)), approximately 20% of women develop placenta mal-perfusion associated complications or thrombotic maternal events. Statins, due to their pleiotropic effects on the endothelium have been proposed to be useful in APS management and have demonstrated to improve pregnancy complications in mouse models of preeclampsia (PE) and APS. Recent publications demonstrated that statins, in particular hydrophilic ones such as pravastatin, are not teratogenic.
Aims: We aim to investigate the clinical use of pravastatin in APS women refractory to classical treatment LDA+LMWH that developed PE and/or severe IUGR.
Methods: The study was approved by the Institutional Ethical Review Committee. All women were informed about the off-label use of pravastatin in pregnancy and signed a written consent. Between 2013 and 2015, 13 women with OAPS did not respond to LDA and prophylactic dose of LMWH (enoxaparin or tinzaparin) and developed PE or severe IUGR. Pravastatin (20 mg/day) was added to their treatment when pregnancy abnormalities were observed (between 22 and 30 weeks).
Results: After pravastatin addition to conventional anticoagulation therapy, placental blood flow and maternal symptoms of PE improved significantly leading to live birth in 100% of the patients. The beneficial effects of pravastatin were observed as early as 10 days, with a mean response time of 14.08 ± 3.25. All babies were born alive and healthy.
Conclusions: Our study indicates that the addition of pravastatin at the time of onset of PE or severe IUGR to conventional treatment is worthy of further assessment in the management of women with antiphospholipid antibodies and preeclampsia. RCT should be organized.

LUP03

Mixing test specific cut-off is more sensitive at detecting *in vitro* lupus anticoagulant inhibition than the index of circulating anticoagulant with multiple APTT and dRVVT reagents

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Background: Guidelines for lupus anticoagulant (LA) detection recommend mixing test interpretation with either a mixing test specific cut-off (MTC) or index of circulating anticoagulant (ICA). We previously proposed MTC was superior to ICA in detecting the *in vitro* inhibition of LA employing a single dAPTT and dRVVT pairing.
Aims: To compare the diagnostic efficacy of MTC and ICA in multiple APTT and dRVVT reagents.
Methods: One hundred-five samples positive for LA in the dAPTT and dRVVT reagent pairing employed for diagnostic testing were then assayed undiluted and in a 1:1 mix with normal pooled plasma with four additional APTT reagents and another dRVVT reagent (dRVVT B) on an automated coagulation analyser. For the diagnostic testing, samples were considered LA-positive if one or both screening test ratios were elevated and corrected by ≥10% with the confirmatory test. Mixing tests were assessed against locally derived MTC ratios (APTT A > 1.07, APTT B > 1.07, APTT C > 1.08, APTT D > 1.04, dAPTT > 1.15, dRVVT A > 1.07, dRVVT B > 1.08) and ICA percentages (APTT A > 12.4, APTT B > 10.4, APTT C > 13.6, APTT D > 12.0, dAPTT > 13.2, dRVVT A > 11.9, dRVVT B > 12.0).
Results: The numbers of elevated screen test in undiluted LA-positive plasma for APTT A, APTT B, APTT C, APTT D, dAPTT, dRVVT A and dRVVT B were 36 (34%), 63 (60%), 43 (41%), 62 (59%), 66 (63%), 74 (71%) and 67 (64%), respectively. MTC positivity was 67%, 78%, 77%, 84%, 46%, 81% and 72%, respectively. ICA positivity was 47%, 25%, 54%, 53%, 42%, 46% and 28%, respectively.
Conclusions: MTC was compared with ICA for the detection of LA in several APTT and dRVVT reagents. In all reagents, MTC was superior to ICA in detecting LA and it is valuable to maximize the diagnostics potential of mixing tests in LA detection.

LUP04

Evaluation of a new formulation dilute russell's viper venom time for detection of lupus anticoagulants

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Background: dRVVT is indispensible in lupus anticoagulant (LA) detection. Several paired screen and confirm reagents are commercially

available yet performance variation exists and therapeutic anticoagulation interference is problematic.

Aims: To compare new formulation dRVVT reagents against two currently available dRVVTs.

Methods: 59 samples from non-anticoagulated patients (NAPs) LA-positive in routine dRVVT (A) (Group 1) were analysed with another commercial dRVVT (B) and the new dRVVT (C); 38 were also dAPTT-positive. Group 2 comprised 15 dAPTT positive/dRVVT negatives from NAPs. Group 3 had 24 VKA anticoagulated patients with APS and/or SLE, LA-positive by one or more of dRVVT, dAPTT and Taipan/ecarin tests. Group 4 comprised 13 patients on rivaroxaban. Group 5 comprised 62 thrombotic patients negative for LA by routine testing and Group 6 comprised samples from 20 VKA anticoagulated, non-APS patients.

Results: dRVVT B detected 56 (95%) of Group 1 LAs and dRVVT C detected 34 (59%) from >10% correction of elevated screen criterion and 46 (76%) via integrated interpretation. dRVVT B and C detected one LA each from Group 2. Accepting interpreting undiluted plasma results is controversial, dRVVT B detected all Group 3 LA in undiluted plasma and 17 (71%) in mixing tests, and dRVVT C, 16 (67%) in undiluted plasma and 11 of 21 with sufficient plasma for mixing (52%). Group 4 results are in Table 1. In Group 5, dRVVT B detected 2 additional LA. From undiluted plasma alone for Group 6, dRVVT A generated 13/15 (87%) false positives, dRVVT B 23/24 (96%) and dRVVT C none.

Table 1 Distribution of multiple LA assay results.

Reason for anticoagulation	dAPTT	dRVVT A	dRVVT B	dRVVT C	TSVT/ET
1 × DVT	+	+	+	-	+
2 × APS, 4 × DVT	-	+	+	-	-
1 × APS, 1 × PE	+	+	+	+	-
1 × APS	+	+	+	+	+
1 × DVT, 2 × PE	+	+	+	-	-

Conclusions: dRVVT C has comparable performance to current reagents in NAPs and LA-positive VKA anticoagulated patients. In view of previously described rivaroxaban-induced false-positives with current dRVVTs, the 6 positives in only dRVVT A and B may have been spurious. These data, plus those on non-APS VKA anticoagulated patients, suggest that dRVVT C is more specific than currently available reagents.

LUP05

Lupus patients with IgM antiphospholipid antibodies are at risk of thrombocytopenia and haemolytic anemia: pooled results from five systematic reviews and meta-analyses

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Background: The association between IgM antiphospholipid antibodies (aPL) and "non-criteria manifestations" is debated.

Aims: To study whether an association exists between IgM aPL-positivity and "non-criteria manifestations" in Lupus patients according to aPL tests and isotypes.

Methods: We conducted five meta-analyses according to MOOSE guidelines. We searched through MEDLINE, EMBASE, Cochrane Library, congress abstracts, and reference lists of eligible studies, without language and publication date restrictions. Two reviewers independently extracted study characteristics and outcome data. Estimates were pooled using random effects models and sensitivity analyses.

Results: Of identified abstracts (mean±SD=1649 ± 689 abstracts per meta-analysis), 31 primary studies were selected in the Pulmonary Hypertension meta-analysis (4480 patients, 410 cases); 28 primary studies were selected in the Livedo Reticularis meta-analysis (3413 patients, 564 cases); 25 primary studies and 1 abstract were selected in the APS-related nephropathy meta-analysis (2128 patients, 482 cases); 83 primary studies and 2 abstracts were selected in the thrombocytopenia meta-analysis (11877 patients, 2399 cases); 38 primary studies and 1 abstract were selected in the haemolytic anemia meta-analysis (7967 patients, 974 cases). Compared with Lupus patients without each non-criteria manifestations, overall pooled odds-ratios according to different aPL tests and isotypes are presented in Table 1.

Table 1 (Abstract LUP05)

Non-Criteria Manifestations	LA	aCL	IgG aCL	IgM aCL	aβ2-GPI	IgG aβ2-GPI	IgM aβ2-GPI
Valve Disease	7 studies, 529 patients 5.88 (2.92–11.84)	14 studies, 983 patients 3.28 (2.06–5.22)	9 studies, 634 patients 5.63 (3.53–8.97)	3 studies, 254 patients 1.67 (0.46–6.05)	NA	NA	NA
Pulmonary Hypertension	15 studies, 1505 patients 1.96 (1.31–2.92)	20 studies, 2628 patients 2.12 (1.44–3.13)	9 studies, 1295 patients 2.64 (1.30–5.36)	4 studies, 912 patients 2.18 (0.80–5.94)	5 studies, 304 patients 1.81 (0.39–8.44)	2 studies, 193 patients 0.94 (0.44–2.01)	NA
Livedo Reticularis	5 studies, 495 patients 4.65 (2.35–9.20)	18 studies, 2678 patients 3.37 (2.45–4.65)	7 studies, 1597 patients 3.26 (2.18–4.88)	3 studies, 531 patients 1.66 (0.97–2.85)	3 studies, 396 patients 3.40 (1.53–7.52)	2 studies, 137 patients 2.90 (0.65–12.92)	NA
APS–Related Nephropathy	9 studies, 827 patients 4.70 (2.36–9.36)	14 studies, 1124 patients 2.88 (1.70–4.90)	4 studies, 355 patients 3.13 (1.09–8.98)	2 studies, 163 patients 1.51 (0.03–88.59)	4 studies, 505 patients 1.66 (0.54–5.11)	2 studies, 245 patients 1.93 (0.27–13.80)	NA
Thrombocytopenia	25 studies, 2724 patients 3.43 (2.59–4.54)	53 studies, 7291 patients 2.31 (1.94–2.75)	27 studies, 4710 patients 1.97 (1.58–2.44)	17 studies, 2440 patients 1.68 (1.34–2.12)	8 studies, 867 patients 2.21 (1.49–3.28)	5 studies, 572 patients 2.04 (1.22–3.41)	3 studies, 388 patients 2.68 (1.44–4.99)
Hemolytic Anemia	12 studies, 2016 patients 4.58 (2.62–8.04)	25 studies, 6639 patients 2.47 (1.86–3.30)	10 studies, 2766 patients 2.27 (1.71–3.00)	12 studies, 4150 patients 2.89 (2.16–3.87)	5 studies, 611 patients 4.39 (1.96–9.83)	4 studies, 471 patients 3.95 (1.46–10.71)	3 studies, 349 patients 3.00 (1.48–6.07)

Results are presented as: number of studies included; number of patients; OR (95% confidence interval). aβ2-GPI: anti-β2-GPI antibodies; aCL: anticardiolipin antibodies; APS: Antiphospholipid Syndrome; LA: Lupus Anticoagulant; NA: non available.

Conclusions: Based on the current literature, risk of haematological manifestations is increased in Lupus patients with IgM aPL. However, IgM aPL are not associated with other non-criteria manifestations.

LUP06

Use of a lupus anticoagulant-resistant routine APTT reagent as a convenient confirmatory test

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Background: Lupus anticoagulants (LA) are associated with an increased risk of thrombosis and laboratory detection is of major importance. Despite guideline recommendations, not all laboratories employ confirmatory tests in their APTT-based testing and instead rely on mixing tests for confirming specificity and presence of LA.
Aims: To assess LA detection with a combined use of routine APTT reagents: one LA sensitive (LS), and the other LA resistant (LR), both from the same manufacturer.
Methods: 105 samples positive for LA with dAPTT and/or dRVVT screen/confirm were assayed with the two APTTs on an automated coagulation analyzer. For the diagnostic testing, samples were considered LA-positive if one or both screening test ratios were elevated and corrected by ≥10% with the confirmatory test. Screen and confirm tests were also performed by mixing 1:1 tested plasma with normal pooled plasma. APTT-LS was employed as screening test and the APTT-LR as confirmatory.
Results: 62 samples out of 105 (59.0%) showed an elevated screen ratio with APTT-LS. From them, 33/62 (53.2%) had shorter values with APTT-LR and could be interpreted as LA-positive. Of those 33 samples, 20/33 (60.6%) were positive with dAPTT and dRVVT, 2/33 (6.1%) with dAPTT alone, and 11/33 (33.3%) with dRVVT alone.
Conclusions: The combination of APTT-LS and APTT-LR was more sensitive to LA activity detected with dRVVT, than with dAPTT. Although APTT-LS showed moderate sensitivity as a screening test, this latter was improved through the combination with the APTT-LR confirmatory assay. This could result from the systemic application of the ≥10% correction cut-off. Interestingly, application of this integrated interpretation allowed identification of 3 more LA patients. APTT confirmatory test improves specificity. Availability of cheap and convenient routine APTT-LR/LS reagents can extend and facilitate LA diagnosis in clinical laboratories.

LUP07

Antiphospholipid antibodies in children with immune thrombocytopenic purpura

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Background: Presence of antiphospholipid antibodies (APA) has been observed in children with immune thrombocytopenic purpura (ITP), but their role for the outcome of disease is controversial.
Aims: The aim of this study was to evaluate the relation between APA and ITP in childhood.
Methods: The study included to children with newly diagnosed ITP, in the period of time between March 2014 and March 2015, at our center. Clinical and laboratory findings, medical treatments, and the course of the diseases were recorded for all patients. At the time of diagnosis, antiphospholipid antibodies including lupus anticoagulant (LA), anticardiolipin antibodies (aCL), anti-beta-2 glycoprotein I (β2GPI) antibodies were studied. The patients who have positive results for APAs at diagnosis were examined again for APAs at 12th week of follow-up period.
Results: Forty children 21 females and 19 males were enrolled the study. APA levels were positive at 12 patients (30%) at the time of diagnosis. Only one patient had all of three APAs, one patient had both aCL IgM and LA, 10 patients had single positive results for APAs. The positivity of β2GPI, aCL and LA were 58.3%, 16.7%, and 8.3% respectively. After 12 week, only 3 of these 20 cases were still positive for APA. There was not a significant difference between APA positiveness and gender groups, platelet counts, and course of disease. The mean age of APA positive patients was significantly higher than in APA negative patients ($P < 0.05$). According to age interval; there was three patients aged between 0-1 year and none of them were APA positive. APA positiveness was found in 7 of 28 (25%) patients aged between1-9 years, and 5 of 9 patients (55.5%) aged between 9-18 years. There was no relationship between APAs and treatment response or outcome of disease.
Conclusions: APAs may be present in children with ITP. It may be related to underlying viral infections or idiopathic, and more common in adolescence. The persistence of APAs may contribute to thrombotic complications in the future.

LUP08

IgG/IgM antiphospholipid antibodies present in the classification criteria of the antiphospholipid syndrome: a critical review of their association with thrombosis

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Background: Despite the update of the classification criteria of the antiphospholipid syndrome (APS), difficulties persist in the identification of patients at risk for thrombosis. Current guidelines include assays detecting IgG/IgM anti-β2glycoprotein I ($a\beta_2$GPI) and anti-cardiolipin (aCL) antibodies, although the relevance of IgM antibodies has been debated.
Aims: Through review of the literature from 2001 to 2014, we aimed to formally establish the thrombotic risk stratification potential of IgM compared to IgG antiphospholipid antibodies (aPL).
Methods: 1128 articles were selected by a computer-assisted search of literature. Of the 177 studies that met our inclusion criteria, the clinical value of IgG/IgM aPL was established through analysis of odds ratios (OR) for thrombosis or percentage positives in the thrombotic population.
Results: By determining the OR/percent positives for the different aPL in the 177 included studies, we clearly found more significant correlations with thrombosis for the IgG compared to IgM isotype. Nonetheless, in a minority of studies, significant associations with thrombosis were found for IgM but not IgG antibodies.
Conclusions: The unavailability of paired results of IgG and IgM for each separate patient hampers evaluation of the added value of isolated IgM positivity. To fully take advantage of results obtained by future studies, we strongly encourage scientists to provide all studied information per patient. We planned a large multi-centre study to investigate clinical associations of isolated/combined positivity for criteria/non-criteria aPL. Importantly, due to the presence of non-pathogenic aPL, quantitative assays are characterized by a high false positivity rate. Optimization of a new functional assay is urgently warranted. The use of thrombin generation assays measuring the whole scheme of coagulation seems promising and may help to eventually reduce APS related morbidity and mortality.

LUP09

Detection of anti-domain I beta-2 glycoprotein I antibodies by chemiluminescence immunoassay in antiphospholipid syndrome diagnosis

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Background: Anti-cardiolipin antibodies (aCL) and anti-β-2-glycoprotein I antibodies (ab2GPI) represent two of three laboratory criteria for detection of antiphospholipid syndrome (APS). The Domain I in anti-β-2-glycoprotein I is a new target for better identification of antibodies and is associated with thrombotic risk in antiphospholipid syndrome.
Aims: The aim of our study was to determine the significance of anti-Domain I beta-2 Glycoprotein I antibodies as a new biomarker for determination of thrombotic risk in antiphospholipid syndrome.
Methods: We detected Domain I ab2GPI on a group of 74 patients with antiphospholipid syndrome diagnosis. All patients had positive antibodies at least in one of the class of aCL and ab2GPI antibodies.
The determination of ACL and β-2-GPI IgG and IgM antibodies was performed by the chemiluminescent assay (HemosIL AcuStar®) as well as anti-Domain I beta-2 Glycoprotein I antibodies.
Due to the missing WHO standard materials, the calibration of method was done by using the reference serum N.E.Harris, Louisville. The results are expressed in GPL/ml. The cut-off for chemiluminescent assay was locally determined on group 40 healthy blood donors.
Results: We detected Domain I ab2GPI positivity at 21 samples in our group. The incidence of thrombotic complications in the entire group was established as 28, 4%, in comparison with a group positive for Domain I ab2GPI with the incidence of thrombotic complications was 57%.
Conclusions: The new chemiluminescent method for detection of Domain I ab2GPI seems to be better compliance with clinical outcome than the actual panel test.
Supported by grant LF-2016-001 and IGA NT 14394

LUP10

Study of anti-cardiolipin (IgG/IgM aCL) and anti-beta2-glycoprotein I (anti-β2GPI) antibodies in patients with ischemic stroke (Ouest of Algeria)

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Background: The study of anti-cardiolipin antibodies is now well accepted and is routinely used in the risk assessment of various conditions associated with thrombosis.
Aims: The aim of the study was to define whether the investigation of aCL is sufficient by itself to evaluate a risk of ischemic stroke.
Methods: We included patients aged 18 to 50 years with ischemic stroke, referred to thrombophilia investigation at Tlemcen university Hospital, from 1 January 2007 to 31 December 2013 (N = 30). Clinical information was obtained from the Neurology division of Tlemcen (Stroke Registry). Laboratory evaluation of AT, PC, free and total PS, activated protein C resistance, fibrinogen, and antiphospholipid antibodies. including aCL, anti-β2GPI antibodies within the first 48 h after admission, and again, in the case of a positive result, at least 12 weeks after the first measurement Moreover, prevalence of thrombophilia were also evaluated and compared to the results obtained in normal controls.
Results: Frequency of aCL and anti-beta2-glycoprotein I (beta2-GPI) antibodies was investigated in 30 patients with ischemic stroke and in 92 controls by ELISA. In ischemic stroke patients IgG aCL were found in 36.7%%, the IgG-IgM-aCL were found in 37%. The levels of both antibodies were higher in patients with ischemic stroke than in controls ($P < 0.01$). In controls, IgM-aCL were positive in 3.3% and IgG-aCL antibodies were negative. The IgG-IgM-anti-beta2-GPI Abs were found in 17% patients. They were negative in controls. The category IIb (aPA IgM/IgG),I (LA and aPAIgG)and IIc (aB2GPI IgM) were found in 20%, 21% and 59% respectively, There was a correlation between levels of aCL and anti-beta2-GPI Abs for both isotypes (P0.03). The sensitivity of anti-beta2-GPI Abs for ischemic stroke was increased when both isotypes were tested.
Conclusions: These results showed that aCL and anti-beta2-GPI Abs could be pathogenetically important for ischemic stroke and that anti-beta2-GPI Abs testing might contribute to a better evaluation.

LUP11

Bone marrow necrosis and pulmonary thrombosis associated with antiphospholipid syndrome

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Background: Antiphospolipid Syndrome (APS) is characterized by recurrent arterial or venous thrombosis. It is rarely associated with bone marrow necrosis (BMN).
Aims: We present a case of APS in a child presenting with pulmonary thrombosis and BMN.
Methods: Thirteen-year-old female presented with pneumonia and pleural effusion. Laboratory studies revealed anemia (7 g/dl), thrombocytopenia (57000/μL), elevated activated partial thromboplastin time (aPTT) and d-dimer. Pulmonary CT angiography revealed acute thrombosis in segmental branch of right pulmonary artery. Pleural fluid examination and cultures, blood cultures were nondiagnostic. Direct coombs test was positive (+++). Viral serology, markers of collagen vascular disease, serum levels of immunoglobulins were normal. Bone marrow aspiration revealed only necrotic cells, while on trephine biopsy; there was extensive infarction of bone marrow with markedly reduced normal hematopoietic cells, without malign infiltration. Sickle cell anemia was ruled out by normal results of haemoglobin electrophoresis. Magnetic resonance imaging (MRI) of abdomen showed bone marrow necrosis in right iliac bones. Lupus anticoagulant screening and confirmation (dRVVT) tests and antiβ2 glycoprotein-1 IgG were strongly positive, anticardiolipin antibody was negative. Other thrombophilic studies were normal.
Results: The patient who presented with pulmonary thrombosis was diagnosed with APS and BMN. Enoxaparin therapy was started for anticoagulation. Prednisolone was given for autoimmune haemolytic anemia. Bone marrow necrosis was still present at the end of 8th week on MRI, but cytopenias were improved. She is in good condition with maintenance therapy of enoxaparin.
Conclusions: BMN is relatively uncommon condition and is most frequently encountered with malignancy, collagen vascular disease, infectious disesase and sickle cell anemia. Our patient has APS which possibly related to severe lung infection. It should be noted that APS can be manifested as BMN even in childhood.

Pediatric/Neonatal Hemostasis and Thrombosis

PED01

Factor XIII levels in children with extensive venous malformations and chronic disseminated intravascular coagulation: a single tertiary centre experience

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Background: Venous malformations are slow flow vascular malformations. These can be sporadic, inherited or syndromic. When extensive they may be associated with disseminated intravascular coagulation (DIC) with a picture of hypofibrinogenaemia, consumption of coagulation factors and thrombocytopaenia. Such patients may have spontaneous bleeding or significant bleeding following surgical procedures. We report findings from a cohort of patients managed at a tertiary paediatric centre.
Aims: To investigate an association with factor XIII (FXIII) levels and bleeding symptoms in children with venous malformations and chronic DIC.
Methods: We retrospectively reviewed blood tests results and clinical notes of patients with extensive venous malformations known to the Dermatology unit, focusing on spontaneous bleeding or perioperative coagulopathy and management.
Results: We identified more than 40 patients with extensive venous malformations. 25% (10) patients had bleeding complications, either spontaneous or peri-procedural. 12.5% (5) patients had fibrinogen < 1 g/l and historically 4 of these patients had severe bleeding complications following procedures despite cryoprecipitate cover. All of these patients had disproportionately low FXIII levels levels (8 - 25 iu/dl) with hypofibrinogenaemia, other clotting factors being normal. This group of patients had FXIII concentrate in addition to fibrinogen replacement for subsequent procedures without any bleeding complications.
Conclusions: We report a finding of disproportionately low FXIII in patients with extensive venous malformations with evidence of DIC and clinically significant bleeding. This is the first case series to report this finding and warrants investigation for similar results in a larger cohort. The pathogenesis behind this laboratory finding will need further research. We propose that FXIII levels be routinely checked in this patient group along with other markers of coagulopathy, as FXIII replacement in these patients may avoid severe bleeding complications.

PED02

Characterization of laboratory coagulation parameters and risk factors for intraventricular hemorrhage (IVH) in extremely premature neonates

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Background: IVH is a significant cause of morbidity and mortality in extremely premature infants. Despite extensive study, little is known about hemostatic disruptions contributing to IVH or normal coagulation parameters < 30 weeks gestational age (GA).
Aims: To characterize coagulation parameters in < 30 week GA neonates and evaluate risk factors associated with IVH.
Methods: In a prospective observational study, cord blood was obtained from neonates 23-30 weeks GA at the time of birth. Neonates were enrolled from 11/2010-01/2015, 507 neonates were screened, 336 consented and 120 enrolled for analysis. PT/INR, aPTT, Factor II, Factor VII, Factor IX, Factor X, Factor XIII, and Factor XIII subunit A were measured. Data was collected as reported to the Vermont-Oxford Network (VON). Clinical variables defined by VON were used to analyze association with IVH.
Results: 24.2% (29/120) neonates enrolled developed IVH. Mean GA and birth weight of all neonates was 27.1 (23.3–30) weeks and 933.8 (375–1800) grams. Table 1 shows coagulation tests by GA. Number per test vary due to plasma availability for evaluation. There was no significant difference among coagulation tests in neonates with/without IVH or by GA. VON clinical variables were analyzed by Fisher exact test and chi-square: for all neonates, chronic lung disease (CLD, $P = 0.032$) and persistent pulmonary hypertension (PPHN, $P = 0.02$) were significantly associated with IVH, and anemia approached significance ($P = 0.054$). Further, CLD, OR 2.5(95% CI 1.07–5.87), $P = 0.035$ and PPHN, OR 6.11(95% CI 1.36–27.41), $P = 0.018$; and anemia, OR 4.05 (95% CI 0.89–18.46), $P = 0.071$ approached significance. 24 additional variables analyzed were not significantly associated with IVH.

Conclusions: We report the largest, prospective study to date of coagulation parameters and risk factors associated with IVH in extremely premature neonates. CLD and PPHN were significantly associated with IVH, and suggest tenuous hemodynamic flow associated with these conditions may alert clinicians to potential IVH.

Table 1 Coagulation Parameters - All Neonates. (Abstract PED02)

Coagulation Test	23–24 weeks GA #Neonates	23–24 weeks GA Mean, Median, (Range), STD	25–27 weeks GA #Neonates	25–27 weeks GA Mean, Median, (Range), STD	28–30 weeks GA #Neonates	28–30 weeks GA Mean, Median, (Range), STD
PT (sec)	10	13.86, 13.35, (10.5–17), 2.04	39	19.18, 14.5, (10.2–80.8), 14.5	32	20.18, 13.9, (10.9–189), 31.3
INR	10	1.35, 1.3, (1–1.7), 0.22	38	1.67, 1.4, (0.9–5.6), 1	31	1.41, 1.3, (1–4), 0.5
aPTT (sec)	10	59.2, 61.5, (30–89), 17.92	36	60.7, 57.5, (29–135), 21.93	31	66.13, 58, (41–192), 32.65
Factor II (%)	9	37.56, 40, (31–45), 5.92	34	37.49, 36, (5–69), 12.56	32	37.94, 38, (23–57), 7.38
Factor VII (%)	10	44.4, 39.5, (26–75), 14.49	38	46.03, 41.5, (5–132), 26.02	35	44.83, 43, (20–93), 15.03
Factor IX (%)	9	33, 33, (19–67), 14.66	31	31.88, 26, (0.13–86), 19.1	28	30.85, 32, (4.8–53), 10.51
Factor X (%)	9	54.33, 51, (35–89), 16	31	61.52, 61, (23–120), 22.74	29	54.72, 54, (29–93), 15.42
Factor XIII (%)	10	45.6, 43.5, (36–72), 10.41	37	41.76, 42, (19–76), 11.45	34	44.28, 44.5, (7.5–70), 13.69
Factor XIII subunit A (%)	9	39.67, 46, (23–57), 13.35	36	39.11, 35.5, (2.5–123), 22.5	34	36.01, 33, (2.5–74), 19.63

PED03

Increased incidence and high recurrence rate of venous thromboembolism in pediatric oncology patients in one single center over 25 years

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Background: Venous thromboembolism (VTE) is a serious complication of cancer and its treatment in children. However, incidence, risk factors and recurrence rate of VTE in this patient group are largely unknown.
Aims: To identify incidence, risk factors and recurrence rate of VTE in pediatric oncology patients.
Methods: A prospective cohort study of consecutive children (0–18 years) with cancer at the Emma Children's Hospital AMC between 1989 and 2015 was done. In children with lymphomas a case control study, matched for age, type lymphoma and gender, was performed to identify thrombotic risk factors. The cumulative recurrence-free survival after first VTE was estimated by the Kaplan Meier method.
Results: A total of 2183 children (♂ :♀ = 1.4:1; mean age 7.53 ± 5.24 years) with cancer were included. Eighty-seven patients developed VTE (3.6%; 95% CI 2.8–4.4). The incidence increased from 0.8% (4/478, 95% CI; 0.0, 1.6%) between 1989 and 1993 to 10.5% (44/421, 95% CI; 7.6, 13.4%) between 2009 and 2013. VTE developed most frequently in patients with ALL (6.5%), lymphoma (5.6%) and germ cell tumor (5.1%). Children with VTE were significantly older (9.34 ± 5.17 years) than patients without VTE (7.46 ± 5.24 years, $P = 0.002$). In children with lymphoma, an association was found between VTE and the presence of central venous catheter, stage IV lymphoma and immobility. Twelve (15.4%) patients developed recurrent VTE. The cumulative recurrence-free survival after first VTE was 89.7%, 87.2%, and 84.6% after 1, 5 and 10 years, respectively.
Conclusions: Over 25 years the incidence of VTE increased in pediatric oncology patients. An association was found between VTE and older age, type of cancer, catheter, immobility and stage IV lymphoma. The recurrence rate is high, which might warrant prophylactic anticoagulation after first VTE during cancer treatment.

PED04

Central venous catheters and pediatric thromboembolism, a report from the children's hospital acquired thrombosis (CHAT) registry

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Background: Hospital-acquired venous thromboembolism (HA-VTE) rates in children are increasing. A multi-institutional collaboration was formed [Children's Hospital Acquired Thrombosis (CHAT)] to create and validate a HA-VTE scoring system and risk stratification scheme to decrease the rate of HA-VTE. Preliminary data from CHAT has determined central venous catheters (CVCs) are the most prevalent risk factor for pediatric HA-VTE.
Aims: To describe CVC characteristics for subjects in the CHAT registry diagnosed with a CVC-associated HA-VTE.
Methods: Subjects aged 0–21 years from three large centers with a HA-VTE from January 2012-present were placed into this IRB-approved registry. Pertinent risk factor data were selected for analysis.
Results: 401 subjects with HA-VTE have been entered into the CHAT registry. 300 (75%) were found to have a CVC-associated VTE, and 8 had more than 1 CVC-associated VTE. The majority of subjects with a CVC-associated VTE were male (58%), median age of 2.5 years (IQR 0.3–13.9), and 123 (41%) were < 1 year of age (Figure 1). In non-CVC-associated VTE subjects, median age was 6.9 years (IQR 1.9–13.5), with only 17 (18%) being < 1 year. The majority of the CVC-VTEs, 170 (55%), were in subjects with peripherally inserted central catheters [PICCs (Figure 2)]. 215(70%) of the CVC-associated VTE subjects had multi-lumen CVCs. Upper extremity lines were placed in 166 subjects (54%) and 129 (42%) were in the lower extremity. The remaining 13 (4%) had CVCs in the umbilical vein or were unknown.

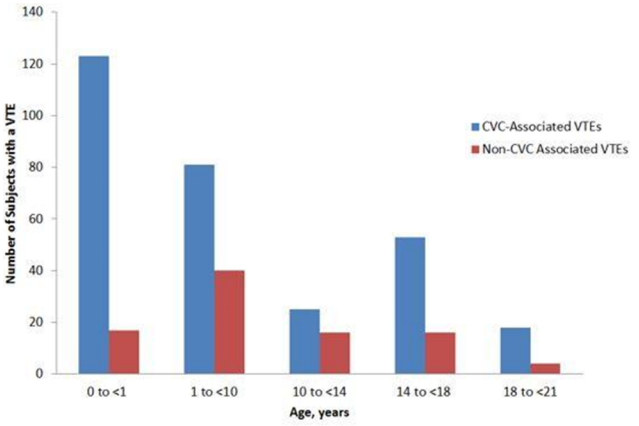

Figure 1. Comparing the age of subjects with and without a central venous catheter-associated venous thromboembolism.

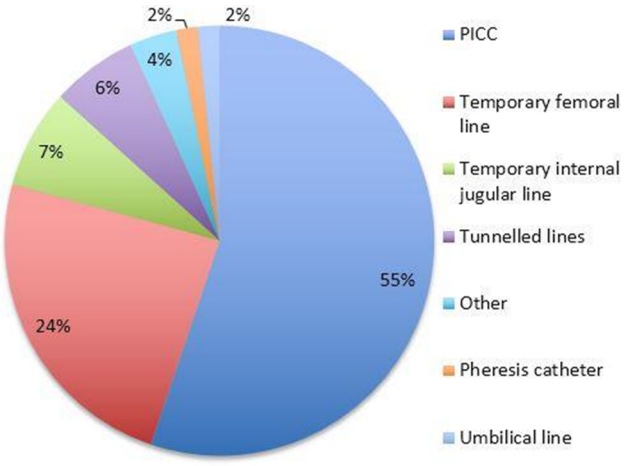

Figure 2. Type of central venous catheter in subjects with catheter-associated venous thromboembolism.

Conclusions: The most prevalent risk factor for pediatric HA-VTE is a CVC. The majority of subjects with a CVC-associated HA-VTE were male, had a PICC, and almost half were < 1 year of age. Subjects also tended to have multi-lumen CVCs placed in the upper extremity. Continued institution and subject recruitment, with the addition of matched controls will occur to complete the analysis of risk factors for HA-VTE, CVC-associated VTE, and formulate predictive models for both.

PED05

Six weeks versus 3 months of anticoagulant treatment for paediatric central venous catheter-related venous thromboembolism

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Background: Central venous catheters (CVCs) are the single most important predisposing factor for the development of venous thromboembolism (VTE) in children. Uniform management recommendations for paediatric CVC-related VTE are not well established. Treatment recommendations suggest anticoagulation for a duration of 6 weeks to 3 months. It has been suggested that shorter treatment durations may provide a similar level of protection against adverse clinical sequelae compared to 3 months.

Aims: The aim of this research project was to retrospectively analyse patients treated for a CVC-related VTE at the Royal Children's Hospital (RCH), Melbourne, to investigate clinical outcomes associated with 6 weeks compared to 3 months of anticoagulation.

Methods: This is a retrospective cohort study. Patients < 18 years of age treated with enoxaparin ± unfractionated heparin for a radiologically confirmed CVC-related VTE at RCH between 2007 and 2014 were eligible. Patients were identified using the RCH pharmacy database, radiological imaging and medical records. Patients were divided into two groups based on the duration of anticoagulation received (6 + 1 week or 12 ± 2 weeks). Data were analysed using Microsoft Excel. Skewed continuous data were expressed as medians (interquartile range). Categorical data were summarised as frequencies and percentages.

Results: Seventy-four patients were included in the study. Higher rates of complete thrombosis resolution were observed in children treated for 6 weeks confirmed by imaging at treatment cessation (39.4%) and long-term follow-up (61.5%), compared to 3 months (11.8% and 9.0% respectively).

Conclusions: Six weeks of anticoagulant treatment for CVC-related VTE may provide non-inferior clinical outcomes compared to 3 months. This study highlights the need for a prospective study focusing on the clinical outcomes associated with different treatment durations following provoked VTE in children.

PED06

Circumcision in children with haemophilia

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Background: Circumcision is one of the most commonly performed procedures in children in most parts of the world and especially in Turkey, however there are few reports which describe circumcision in patients with bleeding disorders.

Aims: The aim of this study is to present our experience in circumcision of children with haemophilia.

Methods: We retrospectively searched the patient records of 79 haemophilia A, and 17 haemophilia B patients. Eleven children with haemophilia were circumcised at Cerrahpaşa Medical Faculty Pediatric Surgery Clinic between 2000 and 2010. Management strategies, complications and outcomes are reviewed.

Results: Among 11 children circumcised; one had haemophilia B (moderate) and ten had haemophilia A (8 severe and 2 moderate). None of the children with haemophilia B had inhibitors whereas 3 with haemophilia A had inhibitors (2 with low-titer and 1 with high-titer). Eight children were on prophylaxis and the rest were on demand therapy. In children with haemophilia without inhibitors, 2-3 doses of factor replacement therapy (15–25/kg/day) was started before surgery and was continued after 24–48 h of circumcision. Fibrinolytics and fibrin glue were also used in these patients. In three hemophilia A patients with inhibitors, by-pass agents were used. Bleeding more than expected was observed in 1 patient without inhibitors and in all patients with inhibitors, however was controlled after factor replacement therapy. Wound healing was normal.

Conclusions: Children with a bleeding disorder can be safely circumcised in a center with experience. In circumcision of children with haemophilia, antifibrinolytics and local hemostatic agents are helpful in decreasing factor doses. If possible, circumcision of children with inhibitor positive haemophilia should be postponed until inhibitors are eradicated.

PED07

Pharmacokinetics and pharmacodynamics of single-dose oral solution of dabigatran given after standard anticoagulant therapy in children, 2–12 years old, with venous thromboembolism

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Background: Dabigatran etexilate (DE) is approved for treatment and secondary prevention of VTE in adults but not those < 18 years (y) old. DE is a pro-drug; the active moiety dabigatran is predominantly renally eliminated. As part of the paediatric program, phase II studies examine DE in three age groups (12–18, 2–12, and < 2 year).

Aims: To assess pharmacokinetics (PK) and pharmacodynamics (PD) of an oral liquid solution of DE in children aged 2–12 year with VTE.

Methods: An open-label, uncontrolled, single-dose study included children2- < 12 year old with VTE (with written, informed consent) who had completed treatment with low-molecular-weight heparin or oral anticoagulation. To account for lower glomerular filtration rates in young children, DE dose was adjusted according to age and weight. Plasma samples at −0.25, 1, 2, 4, 6 and 10 h were collected for PK or PK/PD assessments including diluted thrombin time (dTT), ecarin clotting time (ECT) and activated partial thromboplastin time (aPTT). Safety and tolerability were assessed.

Results: Nine patients (2.5–8 year, 6 male, weight 12–43 kg) received DE (62.5–138 mg). The dabigatran geometric mean (gMean) plasma concentration reached C_{max} 116 ng/mL with geometric coefficient of variation (gCV) 38.6%. The gMean dabigatran plasma concentration fell from 114 ng/mL at 2 h (gCV 37.9%) to 28.2 ng/mL (gCV 37.0%) at 10 h. Mean dTT and ECT values followed the dabigatran concentration profile with mean maximum dTT and ECT of 53.6 s and 79.6 s, respectively. Accordingly, relationships between total dabigatran concentrations and dTT or ECT appeared linear, with a non-linear relationship for aPTT. There were no drug-related adverse events.

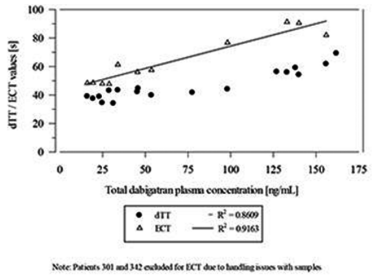

Figure: Relationship of total dabigatran plasma concentrations with dTT (anti-FIIa) values and ECT values after single-dose administration of DE (various doses) in paediatric patients of various ages and body weight (preliminary data).

Conclusions: Across the 2.5–8 year age range, observed variability in dabigatran exposure was moderate when dosed according to the nomogram. The PK/PD relationship was similar to that observed in adults and adolescents. Data from this trial will guide dosing of DE in a paediatric phase IIb/III trial.

PED08

The incidence of thrombosis and PTS in children 2 years after central venous catheter placement

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Background: Asymptomatic thrombosis associated with central venous catheters (CVCs) in children varies in incidence from 5 to 69%. The rate of other long term complications, such as Post Thrombotic Syndrome (PTS), from asymptomatic CVC-related thrombosis is unclear.
Aims: This study determined the frequency of asymptomatic CVC-related thrombosis in children and assessed the incidence and severity of PTS following CVC placement.
Methods: A prospective cohort study recruited children admitted to a paediatric intensive care unit (PICU) requiring a CVC in the jugular or femoral veins. The study was approved by the hospital ethics committee and informed consent was obtained. Each child had a (blinded) ultrasound of the blood vessel in which the CVC was placed during their admission (Phase I). An ultrasound and a PTS assessment using two paediatric PTS tools were performed approximately 24 months following CVC placement (Phase II).
Results: 205 children were recruited. Ultrasounds of 149 children at Phase I determined a 22.1% incidence of CVC-related thrombosis. Two children were symptomatic. Phase II ultrasounds of 115 children confirmed residual thrombosis in 13.2% and vessel wall thickening in 14%. No radiological thrombosis extension or clinical embolization (including paradoxical emboli) occurred in the 123 children assessed at Phase II. A single sign or symptom of PTS was reported in 10 children, and more than one sign or symptom reported in 3 children, however none had any functional impairment. The overall mortality in the cohort was 7.4%, although none died from thromboembolic complications.
Conclusions: Asymptomatic CVC thrombosis is common in children in PICU, however in 2 years of follow up we found no evidence of thrombosis associated mortality and minimal thrombosis associated morbidity. This suggests routine imaging for asymptomatic thrombosis is not warranted and that specific anticoagulant treatment of asymptomatic CVC thrombosis may also be unwarranted.

PED09

Body mass index is associated with symptomatic venous thrombotic events in pediatric oncology patients: a study from maritimes, Canada

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Background: Although a high body mass index (BMI) is generally recognized as a risk factor for venous thrombotic events (VTE) in adults, data addressing its role in pediatric oncology patients is scant.
Aims: To assess the association of BMI with symptomatic VTE (sVTE) in pediatric oncology patients.
Methods: All pediatric oncology patients in 3 Maritime Provinces (Nova Scotia, New Brunswick and Prince Edward Island) are managed through a central tertiary care hospital (IWK Health Centre) in a shared care model with regional provincial physicians. After ethics, approval, the following databases were used to extract study data on patients with sVTE managed at the IWK from January 1st 1995 through Dec 31st 2015:
(i) pediatric oncology hospital database,
(ii) Provincial Cancer in Young People database,
(iii) Electronic medical records,
(iv) Pharmacy database and
(v) Hospital Health records.
Age at cancer diagnosis, gender and BMI of these patients were compared to a control pediatric oncology population without sVTE where complete data sets were available (n = 615).
Results: Over the study period, 58 pediatric oncology patients with sVTE were identified. 53.6% of the sVTE patients were older than 10 years as compared to 35% of the controls (P = 0.014). The gender distribution was similar (P = 0.277) in the 2 groups. The mean BMI of patients with sVTE (21.2 $\pm$ 11.8) was significantly higher compared to control population (18.1 $\pm$ 8.6) (P = 0.001). BMI>25 and BMI>30 were observed in 21.4% (vs. 6.7% controls, P = 0.001) and 12.5% (vs. 2.3% controls, P = 0.001) of sVTE patients respectively. On multivariate analysis adjusting for age, BMI> 25 and BMI> 30 increased odds of sVTE 2.45 (95% CI: 1.1–5.3) and 4.1 (95% CI: 4.1–11) times respectively.
Conclusions: The present study demonstrates that BMI is associated with sVTE in pediatric cancer patients. Further studies are needed to ascertain its role in pathogenesis of sVTE in pediatric oncology patients and determine if targeted intervention may be effective in select high risk population.

PED10

Catheter-related arterial thrombosis in children: a systematic review

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Background: Catheter-related arterial thrombosis (CAT) is increasingly recognized in children. Diagnostic and treatment modalities are based on expert opinions.
Aims: Systematic review of the literature.
Methods: Literature search (1945–2014) on CAT in children aged 0–18 years was performed using PubMed, Embase and Cochrane. Pooled estimates, expressed as percent (%) with 95% Confidence Interval (CI), were generated using the inverse-variance weighted method in random-effect models.
Results: Of 3484 articles, 26 met inclusion criteria (14 prospective studies, 12 retrospective cohort/case series). Of these, 21 (81%) reported on CAT due to indwelling catheter (IC) in the umbilical (18/21, 85.5%), extremity (2/21, 9.5%) or combined arterial locations (1/21, 5%), and 5 (19%) on CAT due to cardiac catheter (CC). 20 (74%) of the 26 studies referred to CAT occurring in neonates. The occurrence rate of CAT was 24% (95% CI=16–32) overall, 12% (95% CI=0–12) for CC and 27% (95% CI=18–36) for IC. Diagnostic methods included ultrasonography (97%, 95% CI = 95–99) and angiography (75%, 95% CI=48–100). Underlying conditions in children with IC-related CAT included respiratory distress syndrome (59%, 95% CI = 51–67), asphyxia (39%, 95% CI = 18–61), infection (29%, 95% CI=12–46) and cardiac disease (22%, 95% CI = 0–51). Cardiac disease was the likely condition in CC-related CAT (96%, 95% CI=91–100). Single or combined treatment modalities included unfractionated/low molecular weight heparin (56%, 95% CI = 28–83), thrombolysis (54%, 95% CI=32–76), thrombectomy (29%, 95% CI=0–57) and no treatment (64%, 95% CI=38–90). Thrombus resolution occurred in 52% (95% CI=36–68), while CAT-related mortality was 19% (95% CI = 7–30).
Conclusions: CAT is an increasingly recognized complication in children, mostly affecting neonates and possibly associated with a non-negligible mortality rate. This systematic review constitutes an essential step to identify the current state and the rationales for further clinical studies on CAT in children.

PED11

Diagnostic challenges and clinical implications in children with congenital bleeding disorders: a developing country perspective

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Background: In developing countries numerous challenges exist in the diagnosis and management of children with congenital bleeding disorders because of limitations in diagnostic facilities and therapeutic options. Lack of awareness, technical expertise, availability of sophisticated equipment leads to misdiagnosis and inappropriate management.
Aims: This study was conducted to assess number and disease characteristics of children with congenital bleeding disorders who were initially misdiagnosed and consequently received inappropriate management.
Methods: This study was conducted at the pediatric outpatient and emergency departments of a tertiary care hospital in Pakistan over a period of 4 years from August 2011 till August 2015. The data was extracted from medical records of patients who failed to improve after initial management and were reevaluated with proper history and further tests. The data comprised age, sex, family history of a bleeding disorder, type and onset of bleeding symptoms initial diagnosis, management given and final confirmatory diagnosis. Complete blood counts, morphology, bleeding time where indicated, prothrombin and activated partial thromboplastin time were done in all patients. For factor assays, von-Willebrand factor antigen and activity, and platelet function tests patients were sent to a referral center due to the non-availability of these tests in our hospital.
Results: Twenty three children were diagnosed as having congenital bleeding disorders. Out of these (n = 6, 18%) children were initially misdiagnosed and managed inappropriately. Three were males and three females. Mean age of those misdiagnosed was 6 years. The details are shown in Table I.
Conclusions: There are chances of misdiagnosis and improper/invasive management if comprehensive laboratory evaluation in conjunction with a thorough clinical evaluation is not carried out in children with congenital bleeding disorders.

Table 1 (Abstract PED11)

Gender	Age	Initial misdiagnosis	Management (After initial misdiagnosis)	Final confirmatory diagnosis
Female	10 years	Immune thrombocytopenic purpura	Corticosteroids,IV immunoglobulins, Splenectomy	Bernard- Soulier Syndrome
Female	12 years	Immune thrombocytopenic purpura	Corticosteroids, IV immunoglobulins, Splenectomy	Von Willebrand Type- II B
Male	5 years	Hemophilia A	Factor VIII concentrates	Von Willebrand type- III
Male	2 years	Hemophilia A	Factor VIII concentrates	Von Willebrand type- III
Female	7 years	Immune thrombocytopenic Purpura	Corticosteroids	Bernard- Soulier Syndrome
Male	5 years	Immune thrombocytopenic purpura	Corticosteroids, IV immunoglobulins	Bernard- Soulier Syndrome

PED12

Risk factors for hospital-associated venous thromboembolism in critically-ill children with cardiac disease undergoing cardiothoracic surgery or cardiac catheter-based therapeutic intervention

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Background: Pediatric hospital-acquired venous thromboembolism (HA-VTE) has dramatically risen in recent years. Children with congenital or acquired heart disease are at particular risk and have not been addressed by recent, novel retrospectively-derived risk scores.
Aims: We sought to develop a risk model for HA-VTE in critically-ill children with cardiac disease.
Methods: We conducted a retrospective, case-control study of children admitted to the CVICU at All Children's Hospital Johns Hopkins Medicine (St. Petersburg, FL, USA) from January 2006–April 2013. We identified cases via ICD-9 codes, and employed case validation via review of radiologic records. Two controls were randomly selected for each case. Associations between putative risk factors and HA-VTE were estimated using odds ratios (ORs) and ninety-five percent confidence intervals (95%CIs) from univariate and multivariate logistic regression analyses. Variables with P-values < 0.1 in univariate analyses were included in the multivariate model. A HA-VTE risk score was developed with weighting based on the relative magnitudes of the individual ORs from the multivariate model.
Results: After adjustment in a multiple logistic regression, length of stay (LOS) >30 days, cardiac catheterization, and major infection were found to be statistically-significant independent risk factors for HA-VTE in these children. An 8-point risk score was developed in which scores of 0–1, 2–6, and 7–8 yielded HA-VTE risks of < 1%, 1-< 2%, and ≥2%, corresponding to conventional thresholds for instituting no prophylaxis, mechanical prophylaxis, and pharmacological prophylaxis (respectively) in hospitalized adults.
Conclusions: LOS >30 days, cardiac catheterization, and major infection are significant independent risk factors for HA-VTE in critically-ill children with cardiac disease leading to the development of a novel HA-VTE risk score in this population. If prospectively validated, this risk score will inform the design of risk-stratified clinical trials of HA-VTE prevention.

PED13

Risk factor assignment concordance and clinical care guideline compliance for prevention of pediatric hospital acquired-venous thromboembolism

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Background: Pediatric hospital-acquired venous thromboembolism (HA-VTE) incidence is increasing but risk factors (RFs) are not clearly defined.
Aims: We evaluated A) agreement of RF assignment from a bedside HA-VTE prevention clinical care guideline (CCG)-derived risk categorization to that assigned on later EHR audits, and B) adherence to CCG prophylaxis recommendations.
Methods: Electronic health records (EHRs) were retrospectively reviewed for 43 children who developed VTE at least 48 h after admission from Aug 2014 to Dec 2015; 35 were identified as high risk [HR]. We compared bedside admission RF selection, performed in 74% of cases, and daily RF assessments, from 83 patient days, to those we identified on later audit to determine accuracy. We also analyzed how often the mechanical (sequential compression boots) and/or chemical (low-dose anticoagulant) prophylaxis recommendations (including hematology consults) in the CCG were followed for patients at HR for VTE.
Results: The bedside admission RF selection matched audits in 100% of cases for 6 of 9 RFs and ranged from 81% to 97% for the remainder (Table 1). Daily RF assignment agreement was also high, except for active infection (76%) and immobility (64%). Immobility risk assignment discordance was eliminated after linkage to Braden Q mobility score in the EHR. Adherence to CCG-suggested prophylactic interventions, or existence of documented or inferred contraindication, was 63% on the day prior to VTE diagnosis (Table 2). This is potentially related to an absence of hematology consults in high risk patients outside the ICU.
Conclusions: We demonstrate the importance of usable HA-VTE RF definitions for proper bedside risk stratification to guide intervention strategies without exposing children to unnecessary thromboprophylaxis. We also show fairly low adherence to intervention suggestions by the risk level algorithm, which may improve after educating providers on the importance of the hematology consult suggested by the CCG.

PED15

Paediatric exposure to intravenous unfractionated heparin

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Background: Unfractionated heparin (UFH) is commonly used for the treatment and prevention of thrombotic events. In paediatrics, UFH is often used preferentially over other anti-coagulants due to its short half-life and reversibility. Recommendations for the use of UFH in children are based on low-grade evidence, and are largely extrapolated from adult studies. Current rates of paediatric exposure to UFH are unknown, as the last audit examining this was performed in 2003.
Aims: This point-prevalence study aimed to identify current rates of exposure to intravenous UFH in a tertiary paediatric hospital, and to describe the population receiving UFH.
Methods: Data was collected over 1 day at a major paediatric hospital in Melbourne, Australia. The medical records of all inpatients were screened to identify any inpatient receiving a UFH infusion. Patient characteristics included age, diagnosis, indication for UFH, clinical team, details of UFH infusion and laboratory monitoring performed. All data was collected from the patient's medical record by 4 researchers using a purpose-designed data collection tool. The tool was piloted prior to the point-prevalence survey and an inter-rater reliability of > 90% was achieved.
Results: A total of 243 patient charts were screened. 4.9% (n = 12) had a UFH infusion in progress. 25% (n = 3) of patients receiving UFH were being treated therapeutically for thrombosis, with the remaining 75% (n = 9) receiving UFH for prophylactic purposes. Half of the patients exposed to UFH were under the age of one, and exposure was most common for patients treated under the cardiology team.

Conclusions: Intravenous UFH remains a commonly prescribed therapy in tertiary paediatrics. Despite the frequency of its use, robust evidence supporting optimal management of intravenous UFH in paediatric practice is lacking. This study justifies the need for future research studies to optimise the management of UFH in children.

PED16

Risk factors associated with paediatric thrombosis: a 5 years retrospective analysis in a tertiary care hospital

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Background: Thrombosis is a rare disorder in paediatric age group as compared to adults. The annual incidence of venous thromboebolism in paediatric population is 0.14 per 10, 000 children. One third of all cases of paediatric thrombosis is catheter related. Whereas prothrombotic disorders comprises of 21% of all cases of paediatric thromboembolism.

Aims: To evaluate the risk factors associated with paediatric thrombosis over 5 years.

Methods: A retrospective 5 years analysis was done from January 2011 till September 2015. Ethical exemption was sought from institutional ethical review committee. Files coded with lower limb deep venous thrombosis, upper limb venous thrombosis, arterial thrombosis, Budd Chiari syndrome, pulmonary embolism, cerebral venous sinus thrombosis and renal vein thrombosis were reviewed for their associated risk factors.

Results: A total of 35 patients aged < 18 years were diagnosed with thrombosis over the period of 5 years from 2011 till 2015. 20 were male, while 15 were female. Out of 35 subjects, only 4 of them were neonates. Overall incidence of hospital acquired thrombosis was 26% (n = 9/35). Table 1 highlights the risk factors associated with thrombosis at various sites.

Table 1 Risk factors for paediatric thrombosis.

SITE OF THROMBOSIS (N= NUMBER OF SUBJECTS)	ASSOCIATED RISK FACTORS(most patients had more than one risk factor)
Lower limb deep venous thrombosis (31.4%) (n = 11/35)	Hospital acquired i.e. Catheter related and prolonged hospital stay (36%, n = 4/11), Malignancy (18%, n = 2/11), Severe sepsis (18%, n = 2/11), Heritable thrombophilia (9%, n = 1/11), Antiphospholipid antibody syndrome [APLA(9%, 1/11)], congenital heart disease (9%, n = 1/11)
Upper extremity venous thrombosis (17%)(n = 6/35)	Catheter related (66%, n = 4/6), Malignancy (17%, n = 1/6), APLA(17%, n = 1/6)
Budd Chiari syndrome (17%)((n = 6/35)	No cause identified (66%, n = 4/6), APLA (17%, n = 1/6), Hyperhomocysteinemia (17%, n = 1/6)
Cerebral venous sinus thrombosis (14%) (n = 5/35)	Meningitis (40%, n = 2/5), Gliomatas cerebri (20%, n = 1/5), Thrombotic Thrombocytopenic Purpura (20%, n = 1/5), Congenital heart disease (20%, n = 1/5)
Pulmonary Embolism (8.5%) (n = 3/35)	Hodgkins lymphoma(33% n = 1/3), Pulmonary Tuberculosis (33%, n = 1/3), Dermatomyositis (33%, n = 1/3)
Arterial thrombosis (8.5%)(n = 3/35)	Purpura fulminans(67%, n = 2/3), Catheter related (33%, n = 1/3)
Renal vein thrombosis (2.8%)(n = 1/35)	? Heritable thrombophilia (1/1)

Conclusions: The commonest risk factor for upper and lower limb thrombosis was iatrogenic (that is prolonged hospital stay and line related). In majority of Patients with Budd Chiari syndrome, no cause was identified. Such patients were screened for heritable thrombophilia during active thrombotic episodes; hence making the diagnosis of heritable thrombolphilia highly questionable. Meningitis was the commonest association for Cerebral venous sinus thrombosis. For Pulmonary Embolism; the causes were multifactorial whereas for arterial thrombosis; Purpura fulminans was the common cause. A prospective study needs to be done to ascertain the actual incidence of thrombosis in paediatric age group along with their management and long term outcome.

PED17

Endovascular stents in children

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Background: Endovascular stents (EVS) have become an increasing accepted interventional procedure in children. There are no studies assessing the role of antithrombotic therapy (ATT) to avoid stent thrombosis(ST) in pediatric patients(pt).

Aims: To describe management and follow-up of a pediatric cohort with EVS for vascular lesions in different organs evaluated at a single center.

Methods: From September 1994 to December 2015, children< 18y of age with EVS who were referred for ATT were studied. Demographic data, clinical indications, stent type, vascular location, ATT, laboratory monitoring, ST and bleeding were retrospectively analyzed. The use of ATT and its duration depended on the stent type and size and the vascular problem or underlying disease. Dose adjustment of ATT was based on TTPA, anti-Xa level, light transmission aggregometry and/or international normalized ratio (INR).

Results: 39pt (23 males, 59%), median age(range) 9.7y (0.1–17.5).Bare-metal stents were implanted in pt with: Cerebrovascular diseases (Arteries: internal carotid,4pt; vertebral 2pt;other,3pt), congenital heart diseases (pulmonary artery,9pt; Blalock-Taussig shunt,2pt; other,2pt), liver transplant (hepatic artery, 5pt; portal vein,4pt), kidney diseases (renal artery,8pt; other,2pt). Luminal EVS diameter was ≤4 mm in 12(31%)pt.UFH was used perioperatively in all pt and enoxaparin in 21 critically ill pt followed by:enoxaparin,1pt; aspirin (ASA),10pt; ASA+clopidogrel,14pt; VKA,14pt. The INR values at the range of 2.0–3.0 were observed for 52% of the follow-up time. 13/24pt were monitored for ASA/clopidogrel, 4 of them required dose adjustment.The median (range) follow-up was 2.9y(0.4–17.9).Five pt died of their underlying diseases,2 pt presented ST and 3pt had major bleeding.

Conclusions: EVS was an effective treatment for a variety of vascular problems in this heterogeneous cohort. A low frequency of stent thrombosis was found probably related to the ATT used according to the EVS features and clinical conditions.

PED18

Pediatric venous thromboembolism: a single centre experience

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Background: Pediatric venous thromboembolism (VTE) and associated complications are rare but increasing. We reviewed our experience of pediatric VTE in 20 years.
Aims: To identify predictors of pediatric VTE, efficacy of treatment and prevalence of complications.
Methods: A retrospective chart review of patients aged 1–18 years with VTE was done. Data were collected on demographics, risk factors, thrombophilia work-up, treatment, and relapse. Neonatal and catheter related thrombosis cases were excluded.
Results: Sixty-five pediatric patients (M/F: 1.5) were recruited. Median age at diagnosis was 8.7 years (range: 1 month–17 years). Thrombotic locations were cerebral veins (n = 34), lower extremities (n = 12), upper extremities (n = 7), DVT & pulmonary embolism (n = 1), splenic and/or portal veins (n = 7), renal vein (n = 2), mesenteric veins (n = 1), and purpura fulminans (n = 1). In 35 patients (53%), a probable acquired risk factor was identified; the most common risks were leukemia, mastoiditis, vasculitis, congenital heart defect and infections. Thrombophilia work-up showed FV Leiden mutation (n = 12), low protein C (n = 12), high FVIII levels (n = 10), low anti-thrombin-3 (n = 4), high homocytein level (n = 3), prothrombin 20210a mutation (n = 3) in 33 patients (50%). Fifty-four patients received anti-coagulant therapy; the majority (n = 49) received low molecular weight heparin (LMWH) and acetylsalicylic acid (n = 4). Three received warfarin, one was on dabigatran study and one received rivoraxabin. Low molecular weight was given as single dose at a dose of 100 μ/kg to all patients. Five had recurrent thrombosis under treatment, one was on dabigatran and the rest were on LMWH. One patient with vasculitis had post-thrombotic syndrome.
Conclusions: The etiology of pediatric venous thromboembolic disease (VTE) is multifactorial, and in most children, 1 or more clinical and inherited risk factors are present. In our experience low dose LMWH may be used with success in pediatric VTE.

PED19

Prevalence and characteristics of epistaxis in saudi adolescents

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Background: Nose bleeding is the commonest bleeding manifestation across general population, especially older children and adolescents, world-widely. It is implicated as a main presentation in many bleeding diatheses. Epistaxis occasionally needs a medical intervention. There is no local data that represents its burden in Saudi Arabia.
Aims: This is an epidemiological study addresses detailed characteristics of epistaxis among young Saudis.
Methods: This cross-sectional study is a part of an ongoing, national project on bleeding disorders. It is based on validated questionnaire approved by International Society of Thrombosis and Hemostasis. Total of 740 first-year undergraduate students in Riyadh were interviewed by qualified health-care providers.
Results: Out of 740 participants (M:F is 1.9:1), 312 (42%) were reported a history of epistaxis (46%male, 36%female), around 30% has significant nose bleeding. Bleeding was encountered more than once monthly in 8.8% and 22.5% bled for >1 min (2.4% for >10 min) and 17%, needed medical attention. Out of the 312 with epistaxis, 84%, 50% and 31% reported spontaneous bleeding, seasonal fluctuation, and epistaxis from both nostrils, respectively. Only 2 subjects were on oral anti-coagulant. Cessation of bleeding was achieved after local compression in 70% and spontaneously in 24%. Only 12% got medical consultation alone, 4% underwent cauterization and 1% reported packing as a medical intervention. No individual received blood transfusion.
Conclusions: We found that epistaxis is a common bleeding symptom among young Saudis with more prevalence in males. Further correlation with laboratory results will refine these data and obtain better understanding of epistaxis role among our patients whom suffering from bleeding disorder.

PED22

Frequency of Factor V leiden and prothrombin mutation in thrombotic events in children in north moravian region

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Background: Factor V Leiden (FVL) 1691G>A mutation and Prothrombin 20210G>A mutation are the two most prevalent causes of inherited thrombophilia and venous thrombembolism (VTE).
Heterozygosity for FVL occurs in 3–8% of European population.
FVL mutation is found in 20–25% of patients with VTE and 50% of patients with familial thrombophilia.
The prothrombin (PT) mutation is affecting 1.5% to 3% of Caucasian Americans. PT mutation is associated with a 3-fold increased risk of VTE.
Aims: To estimate the frequency of FVL and Prothrombin mutation in pediatric patients with thrombembotic history in North Moravian region.
Methods: Retrospective analysis of results of molecular genetic analysis of pediatric patients (< 19 years) with thrombotic events in period 8/2002- 8/2014 was performed. Thrombotic events included were: pulmonary embolism, ischemic stroke and deep vein embolism. Acquired thrombophilia risk factors were not analysed in this study.
Results: Data from 78 patients (pts) were collected. Age span of pts was 2- < 19 years with median 16 yrs. Molecular genetic analysis of FVL mutation status was performed in 77 pts (99%) and PT mutation status in 75 pts (96%).
Heterozygote FVL mutation status had 16 pts (21%). In this group all kinds of thrombotic events were detected. 1 patient (1%) with deep vein thrombembolism was recognized having homozygote mutation status of FVL.
PT mutation was discovered in 4 pts (5%) – in 3 pts with deep vein thrombembolism, 1 patient with ischemic stroke.
None of patients had both FVL and PT mutation.

Conclusions: Results of our study confirm higher prevalence of FVL and PT mutation status even in young population with thrombosis as compared to published data. We consider the mutation status only as one of several causes of thrombotic event.
It is task of other part of analysis to evaluate the additional role of acquired risk factors for occurrence of thrombotic event in pediatric population.

Plasma Coagulation Inhibitors/ Platelet Immunology

PLA01

HIF-2α downregulates tissue factor pathway inhibitor expression in breast cancer

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Background: Abnormal coagulation in cancer patients is initiated mainly by tissue factor (TF), and TF pathway inhibitor (TFPI) plays an important regulatory role in this process. Hypoxic stress is one of the most pervasive physiological stresses in solid tumors. We have previously demonstrated that under hypoxic conditions TFPI expression was transcriptionally repressed by the activation of hypoxia inducible factor (HIF)-1α. However, the role of the HIF-2α remains unclear.
Aims: To explore the role of HIF-2α in the regulation of TFPI expression in breast cancer.
Methods: Gene expression analysis was performed in breast cancer tissue samples. Quantitative RT-PCR, ELISA, Western blot, luciferase reporter gene and ChIP assays were applied in the cell line experiments.
Results: A positive correlation between HIF-2α and TFPI mRNA expression was observed in breast cancer patients. Overexpression of HIF-2α in MCF7 cells resulted in decreased TFPI expression. The activity of TFPI promoter (-1223 to +45 bp) was repressed in response to HIF-2α overexpression. A HIF-2α responsive region was further identified and located in the TFPI promoter region −170 to +21 bp relative to the transcriptional start site.
Conclusions: This study provides evidence that HIF-2α is involved in the regulation of the procoagulant status of breast cancer cells through transcriptional regulation of the TFPI gene. These results suggest that thrombosis in breast cancer patients may correlate with local hypoxic regulation of coagulation factors and their inhibitors.

PLA02

Antithrombin resistance caused by c.1787G>a mutation in prothrombin gene: rare but strong inherited thrombophilia

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Background: Mutation in prothrombin gene c.1787G>A, which leads to Arg596 to Gln replacement in prothrombin molecule (prothrombin Belgrade) impairs binding of antithrombin to thrombin and creates condition of inherited thrombophilia known as antithrombin resistance (*J Thromb Haemost 2013;11:1936*). Although it seems to be very rare, this mutation has been reported so far in several thrombophilic families in Caucasian, Japanese and Indian population.
Aims: In order to get closer insight in biochemical characteristics and clinical expression of this mutation, we investigated all available members of a large Serbian family with this type of hereditary thrombophilia.
Methods: Study was approved by local ethic committee. Out of 17 available members of the Serbian family with c.1787G>A mutation we identified 10 carriers of this mutation. In all of them basic tests of haemostasis have been performed and detailed data regarding occurrence of thrombosis were collected.
Results: All 10 carriers from investigated family were heterozygous for this mutation. Six out of 10 carriers developed thromboembolic events, while 4 were asymptomatic. Mean age of first thrombotic event was 17 (12–36) years. When proposita is excluded from analysis to avoid ascertainment bias, the RR for thrombosis is 5.6, CI 95 0.8–39.0. As mean age of asymptomatic carriers was 18.5 (8–62) years, real RR may be even higher. Majority of thrombotic episodes were deep venous thrombosis, but arterial thrombosis were also recorded. In all carriers, both symptomatic and asymptomatic prothrombin time was prolonged and activity of FII significantly decreased, clearly distinguishing between carriers and noncarriers.
Conclusions: Although rare, mutation c.1787G>A in prothrombin gene represents strong thrombophilia with early occurrence of both venous and arterial thrombosis in significant proportion of carriers. Prolonged PT and decreased prothrombin activity may be simple screening test for this mutation.

PLA03

TFPI levels and correlation with coagulation parameters based on longitudinal observations in healthy subjects

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Background: Tissue factor pathway inhibitor (TFPI) is an important physiological regulator of coagulation. Patients with hemophilia A have been found to have significantly lower full length (fl) TFPI levels than control patients (0.23 ± 0.06 nM vs. 0.36 ± 0.08 nM), suggesting that TFPI deficiency may also confer some protective effect in hemophilia A (Duckers et al., 2008). Inhibition of TFPI improves hemostasis and may become a treatment option for patients with hemophilia including those with inhibitors. In the presented study, data collected over 1 year were used to statistically model the correlation of age, sex, and TFPI levels with coagulation parameters.
Aims: To assess sex-dependent intra- and inter-individual seasonal variation of TFPI.
Methods: Total and full length plasma TFPI levels and coagulation parameters were determined in plasma samples from healthy subjects (18 m/20f) aged 23–64 years collected at monthly intervals. Based on these data, we correlated coagulation parameters (peak thrombin, lag-time, ETP, velocity index) with TFPI levels using linear mixed models, with sex, age, and season as additional predictors.
Results: TFPI levels were shown to increase with age in both sexes, with higher full length as well as total plasmatic TFPI levels observed in males. In the spring and summer months, reduced flTFPI levels were determined in males. No significant effect of sex was observed on coagulation parameters, whereas higher flTFPI significantly reduced peak thrombin, ETP, and velocity index. Accordingly, lag time increased.
Conclusions: Coagulation parameters were significantly influenced by flTFPI only. flTFPI levels in plasma were subject to only slight seasonal variation, while sex and age had a more pronounced effect. Such longitudinal monitoring of TFPI normal plasma level may help guide therapeutic/dosing decisions in patients with hemophilia.

PLA04

Diagnostic issues in antithrombin, protein C and protein S deficiency in the hungarian population: experience of a large thrombosis laboratory

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Background: The molecular genetic background of antithrombin (AT), protein C (PC) and protein S (PS) deficiency is heterogeneous. The different laboratory tests have different sensitivity to these deficiencies and may suffer from interferences that make laboratory diagnosis difficult.

Aims: Our aims were to describe the mutation spectrum of AT, PC and PS deficiencies and evaluation of the laboratory tests in a cross-sectional single center study in Hungary.

Methods: Patients with AT, PC and PS deficiencies diagnosed by routine laboratory methods (Innovance AT, Protein C reagent coagulometric and Protein S Ac from Siemens) between 2007 and 2015 were registered. Sanger sequencing of *SERPINC1, PROC* and *PROS1* was executed and MLPA analysis was performed in sequencing negative cases. Factor V Leiden mutation (FVL) was also detected.

Results: Out of the AT deficients (n = 124) 92 carried the founder mutation, AT Budapest 3 (ATBp3). In addition 17 different causative mutations (8 novel ones) were registered. The anti-FXa based AT activity assay that we used, could detect all type II heparin binding site deficiency, like ATBp3 with high sensitivity. Among the 122 and 132 patients with decreased PC and PS activity in the clotting assays, a high number of FVL carriers (FVL+) were registered. The mutation detection rate for *PROC* was 66% in FVL- and 14% in FVL+ cases, while in the case of *PROS1* it was 41% in FVL- and 19% in FVL+ cases. Nineteen and 14 novel mutations were registered in *PROC* and *PROS1*, respectively and no founder mutation was detected. Type IIb PC deficiency with normal chromogenic PC activity was detected in 8.5%.

Conclusions: The mutation detection rate is practically 100% in AT deficiency, while it is very low, especially in FVL positive cases in PC and PS deficiencies in Hungary. The high rate of mutation negative cases in FVL- suggests that larger gene/chromosome alterations or epistasis should be hunted for. Laboratory assays for diagnosis should be chosen carefully.

PLA05

The performance of thrombin generation assays is compromised at reduced plasma levels of antithrombin III

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Background: A decrease in antithrombin III (ATIII) - a major inhibitor of coagulation - causes a rise in active thrombin. To monitor thrombin generation in human plasma, the calibrated automated thrombogram (CAT) assay is widely used. Hereby, the thrombin-cleavable fluorogenic substrate Z-G-G-R-AMC needs to be in excess. To account for substrate consumption, the fluorescence signal is compared with that of an a-2-macroglobulin thrombin complex.

Aims: We tested the technical feasibility of assessing the effect of ATIII reduction on thrombin generation in human plasma.

Methods: Tissue factor triggered CAT was performed in ATIII deficient plasma with or without an anti-human FVIII antibody used to simulate hemophilia A conditions. The plasma was supplemented with 0.125–2.5 μM ATIII to achieve ATIII plasma levels of 5–100% of normal. Raw data analysis focused on the velocity of fluorescence signal increase.

Results: Reduced ATIII levels induced an apparent increase in thrombin generation in the presence and absence of FVIII. A reduction to 50% ATIII resulted in an apparent 45–55% increase in thrombin peak and ETP values. Importantly, raw data analysis showed that the fluorescence signal reached a plateau after 30–50 min in samples with < 1.25 μM ATIII, due to complete depletion of fluorescent substrate.

At < 1.25 μM ATIII, it is impossible to distinguish between thrombin inhibition and excessive substrate consumption. Samples with ³1.25 μM ATIII did not reach a plateau and showed substrate conversion until the end of the assay.

Conclusions: The results of CAT analysis at low ATIII plasma levels may be subject to misinterpretation due to the reduced physiological inhibition of the generated thrombin. Consequently, the thrombin substrate is consumed, leading to substrate depletion. We therefore conclude that the CAT assay is technically not suitable to assess the effect of ATIII levels < 1.25 μM ATIII corresponding to 50% of normal plasma.

PLA07

Latex-based antithrombin antigen assays can give falsely reduced levels

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Background: We measure antithrombin (AT) antigen (ATAg) when AT activity (AT3C) level is reduced or borderline; a ratio of AT3C/ATAg close to 1.0 suggests quantitative AT reduction (type I) and a low ratio indicates a type II (functional) defect. We developed an automated latex-based ATAg assay using commercial reagents (AT3LA) that gave results in good agreement with the manufacturer's manual procedure (P = 0.5485) and with our manual ELISA assay. AT3LA inter-assay CV was 2.9% at normal level and the reference range for AT3C/AT3LA was 0.89–1.12. Four pregnant subjects had unexpectedly raised ratios of AT3C/AT3LA due to reduced ATAg (60–74lU/dL) with borderline AT3C (81–88 IU/dL).

Aims: To investigate the AT3LA anomaly in pregnancy using two latex-based ATAg assays and comparing both assays with chromogenic, radial immunodiffusion (RID) and ELISA assays.

Methods: Residual citrated plasma (0.0109M citrate) from 22 pregnant subjects (median 18.5 weeks gestation) and 35 subjects not thought to be pregnant (Thromb) that included 15 subjects with low or borderline AT3C (median AT3C 77 IU/dL).

AT3C was measured on an auto analyser using a bovine thrombin-based AT kit. The commercial manual latex ATAg assay was automated (AT3LA). A second latex-based ATAg assay automated (ATLB). Manual ELISA (AT3E) and RID ATAg (AT3RID) assays. Assays were standardised against 3rd SSC. Ratios of AT3LA/AT3E, AT3LB/AT3E, and AT3LA/AT3RID and AT3LB/AT3RID were calculated for both subject groups.

Results: Median pregnancy ratio AT3C/AT3LA was increased in pregnancy (Table 1).

Table 1

Median Ratio of AT activity/AT antigen in each group

Ratio	AT3C/ AT3LA	AT3C/ AT3LB	AT3C/ AT3E	AT3C/ AT3RID
Thromb	1.05	1.04	1.01	1.04
Pregnancy	1.19	1.08	1.02	1.06
Reference range (non-pregnant)	0.89–1.12	-	0.90–1.10	–

In pregnancy, low ratios were seen for AT3LA/AT3E and AT3LA/AT3RID (Table 2).

Table 2

Median Ratio of AT3LA and AT3LB to ELISA and to RID in each group

Ratio	AT3LA/ AT3E	AT3LA/ AT3RID	AT3LB/ AT3E	AT3LB/ AT3RID
Thromb	0.96	0.99	0.97	1.00
Pregnancy	0.86	0.89	0.95	0.98

Statistically significant differences ($P < 0.01$ to $P < 0.001$) were seen with AT3LA assay in pregnancy compared to all other ATAg groups whereas AT3LB did not appear to be affected by pregnancy (Table 2).
Conclusions: Latex-based AT antigen assays vary and AT3LA gave false low levels in pregnancy and could result in misdiagnosis of AT deficiency.

PLA09

Estimates of within-subject biological variation (CV_I) of antithrombin, protein C, Protein S Free and activity, and activated protein C resistance (APCR) during pregnancy

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Background: Physiological changes during pregnancy make interpretation of coagulation parameters difficult. To interpret changes when a pathological process is suspected, the normal course and the estimated within-subject variation (CV_I) of these parameters in healthy pregnancies should be known.
Aims: Describe the course and estimate CV_I of antithrombin, protein C, protein S free and activity and APCR in healthy pregnancies.
Methods: Blood samples were obtained every 4th week during pregnancy and 1-3 days, 2 and 6 weeks after delivery in 20 healthy pregnant women, antithrombin, protein C, protein S free and activity and APCR (with added factor V depleted plasma) were analysed.
Results of coagulation parameters were transformed into multiples of the median (MoM) every 4th week, and further to natural logarithms of MoM, lnMoM, in order to adjust for the physiological changes during pregnancy (achieve a kind of steady-state) before calculating the transformed CV_I by use of ANOVA. Informed consent was obtained and the study was approved by the regional ethical committee.
Results: Antithrombin was stable in most pregnant women, with a decrease in conjunction with delivery for several of them, while protein C showed a larger spread (both increase and decrease). Protein S (free and activity) decreased steadily throughout pregnancy. Both APTT tests used to calculate APCR showed a decrease with no effect on the final APCR ratio. The CV_I based on lnMoM transformation in pregnancy were: antithrombin 3.8% (95% CI 3.3–4.3), protein C 8.4% (95% CI 7.5–9.6), protein S free 11.5% (95% CI 10.3–13.0), protein S activity 9.3% (95% CI 8.2–10.6) and APCR 0.5% (95% CI 0–1.2), which is comparable to non-pregnant women.
Conclusions: Although there is a physiological change in antithrombin, protein C and protein S free and activity in pregnancy, estimation of CV_I after lnMoM transformation is possible. Thus, reference change values can be estimated and used to evaluate if observed changes during pregnancies are as expected.

PLA10

Occurrence of inherited prothrombotic factors, with special reference to genetic alterations in antithrombin III, in North-Indian patients with recurrent pregnancy loss

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Background: Thrombophilia is known to be associated with recurrent pregnancy loss (RPL). Although prevalence of thrombophilic markers reported in RPL varies with ethnic groups, no study has been reported from India yet.
Aims: To assess the frequency of thrombophilic risk markers and evaluate genetic alterations in Antithrombin III (ATIII) deficient RPL patients in India.
Methods: 610 women with history of either recurrent early miscarriage (n = 494) or history of at least one late miscarriage (n = 116) and equal number of healthy female controls were studied. FV_{Leiden} was detected by PCR-RFLP. Plasma levels of PC, PS and ATIII were determined using commercially available kits. Patients with low ATIII levels were selected for polymorphisms analysis (rs2227589, *Pst*I and rs3138521) and DNA sequencing.
Results: 66 patients (10.8%) had low PS, 48 (7.8%) had low PC, 42 (6.8%) had FV_{Leiden} and 30 (4.91%) had low ATIII. Type II ATIII deficiency was more frequent, 20 (66.7%) than type I AT deficiency 10 (33.3%). Polymorphic rs2227589A allele was found in 16 (53.3%) patients with ATIII deficiency and 3 (10.0%) in controls (p value: < 0.001). Subjects with AA genotype (4/30) of this polymorphism had lower ATIII activity levels (mean= 63.35 ± 2.20%) in contrast to those with either GG or GA genotype (mean= 74.25 ± 3.30%) (p value: < 0.0001). *Pst*I (p value: 1) and rs3138521 (p value: 0.5) polymorphisms were not associated with plasma ATIII levels. Sequencing of ATIII gene revealed C-4X mutation which according to our best knowledge has been identified for the first time in RPL.
Conclusions: ATIII deficiency underlying RPL in India is more common than in west. This may be due to rs2227589 polymorphism (AA) which is more common in Indian ATIII deficient patients than in west. Type II ATIII deficiency is also found to be more common in India and its exact pathogenesis needs to be elucidated. The prevalence of PS, PC and FV_{Leiden} is comparable to west.

PLA11

Associations of elevated FVIII levels and APLAs with APCR$^{-FV\ Leiden}$ patients with deep vein thrombosis

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Background: Activated protein C resistance (APCR) is a complex mechanism associated with increased risk of deep vein thrombosis (DVT). APCR is influenced by multiple genetic (FV_{Leiden} and other FV SNPs) as well as acquired risk factors (elevated FV, FVIII and APLAs).
Aims: The aim of the study was to examine the causative factors of APCR in the absence of FV Leiden ($APCR^{-FV\ Leiden}$) in Indian patients with DVT.
Methods: Total 30 (M:F = 18:12) $APCR^{-FV\ Leiden}$ patients and equal number of age and sex matched healthy controls were study subjects. All subjects were typed for Hong Kong, Cambridge and HR2 Haplotype using PCR-RFLP. FV and FVIII activity levels were determined using FV and FVIII deficient plasma kits. LAC was detected by DRVVT method and Anti-β2 GPI and anticardiolipin antibodies were detected by enzyme immunoassay.
Results: FV HR2 Haplotype was seen in 16% of patients and 10% of controls and difference was not statistically significant ($P = 0.554$). Hong-Kong and Cambridge mutations were absent in all patients and controls. 15 patients had elevated FVIII levels (>150%) as compared to 1 in control groups ($P = 0.001$). Mean FVIII levels in patients was 130.6 ± 31.2 IU/dl, compared with 116.8 ± 15.6 IU/dl in the controls and showed statistically significant association with APCR ($P = 0.015$). Apart from FVIII, LAC and Anti-β2 glycoprotein showed statistically significant association with APCR unlike other factors such as FV levels and anticardiolipin antibodies.
Conclusions: Elevated FVIII levels, LAC and nti-β2 glycoprotein were associated with $APCR^{-FV\ Leiden}$ patients with DVT. So these factors can be incorporated as first line investigations in $APCR^{-FV\ Leiden}$ patients.

PLA12

Identification and significance of anti-MPL autoantibody in immune thrombocytopenia

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Background: Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by enhanced platelet destruction and impaired thrombopoiesis. Thrombopoietin (TPO) identified as a megakaryocyte colony-stimulating factor is recommended as the second-line treatment option and has shown clinical efficacy in ITP.
Aims: Whether antibody against TPO receptor c-Mpl (anti-Mpl antibody) presents in ITP patients has not been addressed. The present study aims to investigate the presentation and clinical significants of anti-Mpl antibodies in ITP.
Methods: Plasma of 148 ITP patients and 52 healthy control individuals were collected for detection of anti-Mpl autoantibody and TPO level by enzyme-linked immunosorbent assay (ELISA). Modified MAIPA was performed to detect anti-platelet antibodies. Clinical features, megakaryocyte counts in bone marrow, TPO level and anti-platelet antibodies were compared between ITP patients with or without anti-Mpl autoantibodies.
Results: Anti-Mpl antibody was detected in 38 ITP patients (26%), but in none of the healthy controls. Patients with anti-Mpl antibodies had lower TPO levels in plasma compared with those patients without the antibodies ($P < 0.05$), but higher than healthy controls. The patients with anti-Mpl antibody showed lower megakaryocytes and platelets count ($P < 0.05$) than anti-Mpl antibody negative patients before treatment initiation. Part of anti-Mpl antibody positive patients possessed anti-platelet antibodies simultaneously (78%). Patients with anti-Mpl antibody had higher response rate to TPO treatment than those without the anti-Mpl antibody. Moreover, the anti-Mpl antibody was not detected or the antibody titer decreased in portion of ITP patients after treatment.
Conclusions: Autoantibody to c-Mpl was detected in a subset of ITP patients and it associates with TPO level, bone marrow megakaryocytes and PLT counts. The anti-Mpl antibody might be a potential indicator for diagnosis, treatment and prognosis of ITP.

PLA13

Decreased miR-142-3p promotes survival of hyperreactive B cells by targeting baff receptorin immune thrombocytopenia

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Background: Immune thrombocytopenia (ITP) is a common bleeding disorder but theunderlying disease mechanism remains poorly understood.MicroRNAs (miRNA) are importantmolecules in regulating gene expression. However, the functions of altered miRNAsin ITPare still largely unknown.
Aims: The goal of this study is to explorethe potential functions of miRNAs in the immunopathogenetic mechanism of ITP.
Methods: The altered plasma miRNA profile was screened by miRNA microarray and validated by quantitative real-time PCRfrom 66 ITP patients and 58 healthy controls. CD-41 labeled platelet microparticles were isolated and used to identify the origin of the altered miRNAs.The functions of miR-142-3p on B cell were tested in Raji cell line by transfecting mimics. Bioinformatic analysis was performed to predict the target genes of miR-142-3p. Dual luciferase reporter assay was performedin 293T cell to identify the targets of miR-142-3p. Passive ITP mouse model was also established to explore the function of miR-142-3p *in vivo*.
Results: A total of 53 miRNAs were found to have higheror lower expression levels (>1.5 fold, $P < 0.05$) in ITP patients compared with that in healthy controls. miR-142-3p were confirmed to be downregulatedin ITP patients and were mainly derived from platelet microparticle.miR-142-3p targeted BAFF receptor (TNFRSF13C) in B cell and promoted BCR-mediated cell death.
Conclusions: Our results indicate that decreased miR-142-3p might benefit the survival of hyperreative B cells and contribute to the pathogenesis of ITP.

PLA14

The outcome of eltrombopag therapy in immune thrombocytopenia: a multicenter study from Turkey

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Background: Immune thrombocytopenia (ITP) has a complex pathogenesis. Thrombopoietin receptor agonists, like eltrombopag, are used in the treatment of refractory ITP.
Aims: In this retrospective multicenter study, we aimed to analyze the efficacy, safety and outcome of eltrombopag in adult ITP patients.
Methods: The demographic and clinical data were obtained from medical records. 89 (59 F, 30 M, median age:48.5, range:18–80) ITP patients from 15 hematology centers were included. Ethical consent was obtained.
Results: Eltrombopag was used as second-line therapy in 6 patients; third-line in 26; fourth-line in 28; fifth-line in 20;and as further lines of therapy in 9 patients. 87 patients had used steroids before eltrombopag; and 15 had used rituximab. Splenectomy before eltrombopag had been performed in 44 patients.The median platelet count at the beginning of eltrombopag treatment was 13000/mm^3 (range: 0–42000). 33 ITP patients had minor bleeding symptoms. Eltrombopag achieved complete response (CR) in 71 (79.8%); partial response (PR) in 8 (9%) patients; 10 patients were unresponsive. 59 of 79 patients who obtained CR or PR were followed up for >3 months. There were 14 relapses within a median of 6 months (range: 3–60). The frequency of relapses was significantly less in female ITP patients (14.6% vs. 40%, $P = 0.049$). Relapse-free survival in females also tended to be longer (not reached vs. 16 months, $P = 0.068$). There were some side effects in 37 (41.6%) patients: Headache and myalgia (12 patients each); minor infection, cutaneous rash, fever, and pruritus (4 patients each); hypertransaminasemia (3 patients); diarrhea (2 patients). Thromboembolic events after eltrombopag were observed in 8 patients: 4 died. The causes of mortality were cerebrovascular event, severe infection, malignancy, unknown (one case each).
Conclusions: Eltrombopag seemed to be effective in ITP patients refractory to conventional drugs. Interestingly, the frequency of relapses was less in females.

PLA16

Platelet proteomics profiling in transfusion adverse events

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Background: Blood platelets destined for transfusion release panoply of molecules during preparation and storage. The leukoreduction process made the transfusion safer but did not completely abolish the adverse events.
Aims: The rationale of this study is to identify potential proteins in Platelet Components (PCs) involved in acute transfusion reactions (ATR).
Methods: Supernatant from leukodepleted PCs was sampled from 5 PCs implicated in adverse events and 5 PC matched controls.
We performed a Label-Free quantitative analysis using an LC-MS/MS method: LC system (Dionex, Amsterdam, The Netherlands) coupled to an Electrospray Q-Exactive quadrupole Orbitrap benchtop mass spectrometer (Thermo Fisher Scientific, San Jose, CA). Subsequently, data were searched by SEQUEST through Proteome Discoverer 1.4 (Thermo Fisher Scientific Inc.) against the Homo sapiens Reference Proteome Set (Uniprot version 2015-07; 68482 entries). Raw LC-MS/MS data were imported in Progenesis QI 2.0 (Nonlinear Dynamics Ltd, Newcastle, UK) for peptide quantification and statistical comparison (ANOVA test).
Functionnal analysis was performed using Ingenuity Pathway Analysis software (IPA, Ingenuity Systems, Redwood City, CA).
Results: 323 proteins were identified in our samples from which 123 were differentially expressed between the two studied groups. These 123 proteins have mainly inflammatory functions and are involved in many biological signaling pathways. The most relevant 10 signaling pathways are: acute phase response signaling, integrin signaling, clathrin-mediated endocytosis signaling, actin cytoskeleton signaling, coagulation system, LXR/RXR activation, remodeling of epithelial adherent's junctions, complement system, glycolysis I and intrinsic prothrombin activation pathways.
Conclusions: The proteomic study of PC supernatants that have induced an ATR could help advancing our understanding of ATR process and would provide leads to help preventing adverse reactions in transfused patients.

PLA17

Sensitive and specific functional flow cytometry assay for the rapid diagnosis of heparin-induced thrombocytopenia and thrombosis (HIT)

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Background: Reliable diagnosis of HIT is mandatory for patient management. Commonly used antibody-detecting immunoassays carry certain limitations compared to functional assays which determine antibody-mediated platelet activation. However, current functional assays are limited by feasibility.
Aims: To assess the sensitivity and the specificity of a simple functional flow cytometric assay (FCA) as compared to a widely used immunoassay, and in correlation with HIT clinical presentation.

Methods: Consecutive samples from patients clinically suspected for HIT were tested by the PF4/H-PaGIA immunoassay and the functional FCA which determines the capacity of the patient′s serum to induce platelet activation in the presence of heparin. The assays results were correlated with the clinical HIT presentation based on the 4Ts score.
Results: Of 649 samples tested, 99 (15.3%) were positive by the H/PF4-PaGIA and 31 (4.8%) by the FCA. Following sample dilution to 1:32, the H/PF4-PaGIA-positive results decreased to 29 (4.5%), constituting a 70.6% reduction, whereas the number of FCA-positive results remained consistent. Forty normal samples were all negative by both assays. The overall agreement between the assays following dilution was 93.1% for positive (sensitivity) and 94.3% for negative results (specificity). Overall, the FCA showed significantly higher correlation with the clinical presentation of HIT (4Ts score) compared to the PF4/H-PaGIA (ROC-plot analysis, AUC 0.93 vs. 0.63, $P < 0.001$). At a cut-off level of 92% sensitivity, the respective specificity of the FCA was 96%.
Conclusions: Our findings suggest that the present functional FCA is practical for routine daily use, providing reliable results for initial diagnosis, as well as confirmation of HIT.

PLA18

Comparison head-to head of two functional assays in the assessment of heparin-induced thrombocytopenia

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Background: Diagnosis of Heparin-induced thrombocytopenia (HIT) is difficult to achieve especially in cardiac surgery and requires laboratory confirmation using functional tests.
Aims: The aim of the study was to compare the performance of 2 functional assays: serotonin release assay (SRA) and heparin-induced platelet activation test (HIPA) in patients suspected of HIT in cardiac surgery and medical setting.
Methods: 88 patients suspected of HIT (63 after cardiac surgery and 25 in medical setting) were included between 2010 and 2015. Criteria of HIT suspicion were: no other cause of thrombocytopenia than HIT ± thrombotic event, positive anti-PF4/heparin IgG (OD>0.5 Zymutest), recovery of platelet count after discontinuation of heparin treatment. Diagnosis was confirmed by HIPA (if patient's plasma activated at least 2 donor washed platelets in the presence of 0.2 or 0.5 IU/ml of heparin but not at 0 and 50 IU/ml heparin) and/or SRA (release ≥20% in the presence of 0.1 or 0.5 IU/ml of heparin with no release at 0 and 10 IU/ml heparin from washed platelets of at least one donor). Two senior hematologists established final clinical diagnosis.
Results: Among the 88 patients, 44 (50%) had a final diagnosis of HIT based on HIPA and/or SRA positive results. Sensibility and specificity for SRA were 0.93 and 0.91, and for HIPA 0.85 and 1, respectively. Positive and negative predictive value of HIPA and SRA were very similar.

Table 1

	HIPA	SRA
Sensitivity, % (95% IC)	84.6 (69.5–94.1)	92.9 (80.5–98.5)
Specificity, % (95% IC)	100 (89.4–100)	90.9 (75.7–98.1)
Positive predictive value % (95% IC)	100 (89.4–100)	92.9 (80.5–98.5)
Negative predictive value % (95% IC)	84.6 (69.5–94.1)	90.9 (75.7–98.1)

In 8 patients with HIT diagnosis, discordant results were observed : SRA was the only positive test in 5 out of the 8 and HIPA in 3 others. In 2 patients without HIT confirmed, SRA was the only positive functional test. No patient had only HIPA positive test in this group.
Conclusions: We observed good and similar performance of HIPA and SRA. Interestingly, sensibility and specificity seem better in cardiac surgery than medical setting. Comparison head-to-head of HIPA and SRA demonstrates that HIPA could be used to confirm the HIT diagnostic in all clinical settings.

PLA19

Improved regulatory B-cell in adult patients with chronic immune thrombocytopenia treated with rapamycin

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Background: Immune thrombocytopenia (ITP) is a common hematologic disorder characterized by isolated thrombocytopenia. Regulatory B cells (Bregs) are a distinct B cell subset with immunoregulatory properties. Rapamycin, as an immunosuppressant, has been used safely and effectively to treat various immune-med-disordors. However, the effect of rapamycin on human Breg cells in cITP were not explored.
Aims: This study was to investigate the role of regulatory B cells before and after rapamycin treatment, and its association with Tregs in chronic ITP patients.
Methods: Rapamycin therapy was applied in a total of 56 patients with cITP who failed with standard dose corticosteroids or splenectomy.The frequencies of Breg cells (CD19 + CD24(hi)CD38(hi)) and CD4(+)CD25(hi)Foxp3(+) Treg cells were analyzed by flow cytometry. The levels of interleukin-10(IL-10) and transforming growth factor-β (TGF-β) were measured using ELISA.
Results: Complete response and overall response were observed in 17 (30.4%) and 29 patients (51.8%), respectively. Compared with the control group, a significant decreased level of Breg cells, Treg cells, IL-10 and TGF-β expression were found in cITP patients ($P < 0.05$). Increased percentage of Breg cells, Treg cells, IL-10 level and TGF-β expression were present in cITP responders after rapamycin treatment ($P < 0.05$). There was a positive correlation between Breg cells and Treg cells in cITP both before and after therapies.
Conclusions: Our findings indicate that rapamycin therapy could induce significant changes of Breg cells to induce response in patients with cITP.

PLA20

Slow responders to *Helicobacter pylori* eradication in adult japanese patients with immune thrombocytopenia

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Background: Several studies have indicated that platelet recovery occurs in a subgroup of immune thrombocytopenia (ITP) patients after successful eradication of *Helicobacter pylori (H pylori)*. Most responders present with a rapid platelet increase within a couple of

weeks, however we identified that some *H pylori*-infected ITP patients showed a very slow platelet response after eradication without any additional treatments.
We previously reported that FcγRIIB polymorphisms are closely related with susceptibility to *H pylori*-infected ITP and platelet responses after eradication.
Aims: In this study we tried to elucidate the mechanisms how the *H pylori* eradication increase platelet counts of H pylori-infected ITP patients.
Methods: We examined FcγRIIB 232I/T polymorphisms and measured the number of circulating anti-GPIIb/IIIa autoantibody producing B cells using peripheral blood samples from *H pylori*-infected ITP patients.
Results: We identified five slow responders in our cohort. The platelet counts gradually increased by about 10-20x10^9/L a month, and it took more than 3 months to reach the peak level after successful eradication. They never received any other treatments for ITP over this time period.
Analysis of the FcγRIIB 232I/T polymorphisms revealed that all the slow responders are non-T carriers with the FcγRIIB 232I/I genotype, except one T carrier (I/T genotype), while non-T carriers only account for about half of the Japanese *H pylori*-related ITP patients. The number of circulating anti-GPIIb/IIIa autoantibody producing B cells decreased several months after successful eradication along with the apparent platelet recovery in all slow responders.
Conclusions: These different responses to *H pylori* eradication suggest that multiple processes are responsible for platelet recovery in ITP patients.
Accumulation of such cases are required to elucidate the pathogenesis of the *H pylori*-related ITP.

PLA21

mTOR staining in bone marrow and spleen tissues of immune thrombocytopenia patients is associated with shorter relapse-free survival

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Background: Immune thrombocytopenia (ITP) is an autoimmune disease characterized by a low peripheral blood platelet count.Intracellular signaling pathways are active and play important roles in the pathogenesis of ITP.
Aims: We evaluted activity of Syk and AKT pathways by immunohistochemical staining of bone marrow and spleen tissues of ITP patients with Syk, mTOR, and 4E-BP1 stains; and investigated whether they were associated with clinical features and treatment outcome.
Methods: The study included 59 ITP patients (45 F,14 M, median age:43, range:18-87). Secondary thrombocytopenias were excluded. Demographic, clinical features and outcome of patients were obtained from medical charts. Bone marrow (BM) biopsies (29 patients) and splenectomy tissues (30 patients) were stained with Syk, mTOR and 4E-BP1. The study was approved by the local ethical committee.
Results: At initial diagnosis, 40 patients had platelet counts < 30000/mm^3. First-line treatments were: steroids in 47, IVIG in 2 patients.Complete response was obtained in 37 patients; partial response in 13; six patients were nonresponsive. Splenectomy was performed in 33 patients:CR or PR were obtained in 30.The median follow-up of all patients was 48 months (3-156).After first-line therapy, relapse occurred in 33 patients. After splenectomy, 6 patients relapsed. Syk staining was observed in 80% of spleen and 96.6% of BM samples. mTOR staining was positive in 80% of spleen and 27.6% of BM tissues. 4E-BP1 was positive in all spleen and BM tissues.In univariate analysis according to Kaplan-Meier analysis, cytoplasmic mTOR positivity in BM was associated with shorter relapse-free survival after first-line treatment (median:64 vs. 123 months, $P = 0.04$).Cytoplasmic mTOR positivity in spleen tissue was also associated with shorter relapse-free survival after splenectomy (median 34 vs. 233 months, $P = 0.045$).
Conclusions: In our ITP patients, mTOR expression in spleen and BM was associated with shorter relapse-free survival.

PLA22

Integrin variations: molecular dynamics and allostery

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Background: The integrin αIIbβ3 is a fibrinogen receptor involved in platelet aggregation. Variants of αIIb and β3 genes are involved in 2 bleeding disorders: Glanzmann thrombasthenia (GT), a hereditary disorder caused by defects in αIIbβ3 expression/function; Fetal/neonatal alloimmune thrombocytopenia resulting from immunization to the allele not carried by a patient during pregnancy / transfusion. Most variants result from amino acid substitutions (aa) in αIIb or β3. Although a lot of them have been identified, their impact on the αIIbβ3 structure remains to be determined.
Aims: A specific protocol of production and analysis of molecular dynamic simulations was set up to study their structural effects.
Methods: To shorten computational time, the complex was segmented in compact sub-domains usable in MDs. Trajectory were analyzed with classical and innovative methods based on a structural alphabet (Protein blocks) allowing studying very local modifications. DMs simulations were done using a structural model of the αIIbβ3 ectodomain (Protein Data Bank code 3FCS) modified according to ***Jallu et al, 2012 et 2014***.
Results: A preliminary study was done using 7 GT variants of the αIIb Calf-1 domain. Our strategy allowed doing 11 independent MDs simulations for a cumulated time of 850 nsec for each variant and the wild type. Surprisingly, compensatory mechanisms moderated local effects of aa substitutions on structure and dynamic. More unexpected was the discovery of distant allosteric effects, some being common to all variants.
Conclusions: This study validated our model, long MDs simulations and their analyses. We discover local mechanisms of structural compensations but also allosteric effects. Other αIIbβ3 sub-domains (and sub-domain associations) and their variants are currently under study. We aim at confirming the observed allosteric mechanisms and at determining if such processes are actors of the pathology or only side-effects. In the end this method aims at being used to study all integrins and their variants.

PLA23

Can heparin-induced multiple electrode aggregometry improve heparin-induced-thrombocytopenia (HIT) diagnosis in the intensive care unit setting? a prospective study

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Background: HIT suspicion in intensive care units (ICU) is frequent but its incidence represents <1% of all thrombocytopenia. Prompt

diagnosis of HIT and fast adopted therapeutic decisions are crucial. Assays easy-to-perform and widely accessible to non-expert laboratories could optimise HIT suspected patients in ICU diagnosis.

Aims: We sought to determine whether Heparin-induced multiple aggregometry (HIMEA) is a reliable method for HIT diagnosis in ICU setting.

Methods: The study included eighty one consecutive blood samples suspected for HIT, addressed to the hematology laboratory from ICU of various hospitals during a 6 month period. A 4T score was performed for every patient. The following tests were realised: ELISA GAM (Zymutest Hyphen Biomed), HIMEA (Multiplate analyser, Roche Diagnostics) Serotonin release assay (SRA). HIT was defined as positive when: 4 T'score $\geq$ 4, platelet count recovery after stopping heparin treatment in favour of HIT diagnosis, positive SRA and ELISA GAM assay. HIMEA was considered positive when a typical sigmoid aggregation curve was present after addition of 1 anti- Xa IU/ml UFH IU/ml and a decrease in AUC value equal or higher to 50% in the presence of 100 anti-Xa UFH. Sensitivity, specificity, positive predictive value (PPV), and the negative predictive value (NPV) depending upon HIT diagnosis were determined for HIMEA.

Results: 4T score was low in 51 patients, intermediate in 27 patients and high in 3 patients. Sepsis was present in 44 patients and 13 underwent extracorporeal circulation (CEC). HIMEA showed an excellent VPN and sensitivity at 100%. VPP and specificity were at 78% and 97% respectively. Agreement with SRA was also excellent. Two patients undergoing CEC showed discordant results (Table below).

Table HIMEA results against HIT diagnosis.

	HIMEA+	HIMEA-	Total
HIT+	7	0	7
HIT-	2	72	74
Total	9	72	81

Conclusions: HIMEA is a robust and easy to perform functional assay that can be used in routine clinical practice for HIT diagnosis in ICU setting. Further evaluation in a larger population is needed.

PLA24

Very low incidence of heparin-induced thrombocytopenia in pediatric patients, even in those with a high pretest probability

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Background: Heparin-induced thrombocytopenia (HIT), a prothrombotic adverse reaction to heparin, can occur in children. However, reliable studies in pediatric patients are sparse, since most did not include a platelet activation assay to confirm the diagnosis of HIT.

Aims: To clarify the clinical and serological characteristics of pediatric patients clinically suspected of having HIT.

Methods: A multicenter cohort study of patients clinically suspected of having HIT was conducted with a nationwide registry. We analyzed 401 consecutive patients enrolled at 181 hospitals, who were divided into two groups: pediatric (age < 20) and adult (aged $\geq$ 20). We compared serological assay results and clinical presentations, such as the pretest probability of HIT based on the 4Ts score and the incidence of thromboembolic events (TEEs), between the groups. HIT was confirmed only when the washed platelet activation assay (WPA) was positive.

Results: There were 17 and 384 patients in the pediatric and adult groups, respectively. None of the 17 pediatric patients had a positive WPA. In contrast, 107 adults (28%) had a positive WPA (P=0.011). Anti-PF4/heparin IgG was detected by ELISA in 154 (40%) adult and 4 (24%) pediatric patients (P=0.17). However, these 4 pediatric patients with positive ELISA did not have TEEs. In contrast, among 107 adult HIT patients confirmed with a positive WPA, 56 (52%) developed TEEs. The incidence of TEEs was similar among pediatric (24%) and adult (30%) non-HIT patients (P=0.59). There were no significant differences between the pediatric and adult groups in the proportion of patients with 4Ts score $\geq$ 4 (53% vs. 61%, *P*=0.49) and percent reduction in platelet count (70% vs. 68%, *P*=0.71).

Conclusions: HIT is rare in pediatric patients, even in those with a moderate or high pretest probability of HIT. Our results underscore that detection of anti-PF4/heparin IgG by ELISA without WPA confirmation may lead to over-diagnosis of HIT, especially in children.

PLA25

A rapid IgG specific lateral flow immunoassay demonstrates superior diagnostic performance to platelet Factor 4/Heparin-Particle Gel immunoassay in screening for heparin-induced thrombocytopenia

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Background: Heparin-induced thrombocytopenia (HIT) is a serious complication of heparin administration caused by activating IgG antibody (Ab) to platelet factor 4/heparin complex (PF4-HEP). Diagnosis of HIT is clinical challenging. Immunoassays detecting Ab to PF4-HEP used as screening tests are not very specific. The high false positives can result in unnecessary confirmatory tests, which are not generally available, as well as avoidable changes in anticoagulants.

Aims: This study aims to compare diagnostic performance of two distinct assays detecting IgG-specific vs. polyspecific Ab to PF4-HEP in HIT screening.

Methods: Consecutive samples suspected for HIT during 2007-2015 were sent for analysis. The rapid IgG-specific lateral flow immunoassay (IgG-LFI) and the rapid particle gel immunoassay (PaGIA) detecting all Ab classes to PF4-HEP were performed. Samples yielding positive results from either test were confirmed by the in-house platelet aggregometry measuring heparin-induced platelet aggregation (HPA) using platelet-rich plasma from healthy donors with known reactive platelets. In addition, clinical courses of the patients were also reviewed to ensure that HPA test results were accurate.

Results: Among 100 suspected for HIT, there were 10 IgG-LFI+ and 31 PaGIA+. There were 8 confirmed HIT, which all yielded positive both tests. Both assays showed 100% sensitivity and negative predictive values, while IgG-LFI and PaGIA showed 97.8% vs. 75% specificity and 80% vs. 25.8% positive predictive value, respectively. The receiver operating characteristic curve analysis showed that IgG-LFI was statistically superior to PaGIA in HIT screening with the area under curve of 0.989 vs. 0.875 ($P < 0.0001$), respectively. The IgG-LFI avoided unnecessary confirmatory testing in 91.3%.

Conclusions: The IgG-LFI displayed superior diagnostic performance to the PaGIA in screening for HIT and reduced medical costs as well as risks of inappropriate medical management.

PLA26

The high frequency of antibodies against the platelet Factor 4/Heparin complex in patients with lung transplantation is not associated with thrombotic events

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Background: The presence of antibodies directed against a complex of Platelet Factor 4 and heparin (PF4/H) is associated with an increased thrombotic tendency. Recently, it was described a high frequency of PF4/H Ab after liver transplantation, despite a strong immunosuppressive treatment.

Aims: Normal 0 21 To analyze the incidence of PF4/H Ab in patients with bipulmonary transplantation (BPT) and to identify a possible consequence on thrombotic events.

Methods: Citrated plasma of 59 patients [cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD)] were analyzed at 3 times: before the BPT (T1), within 10 and 17 days (median 14 days) after BPT (T2) and at least 3 months after BPT (T3). Patients were treated by a triple immunosuppressive regimen (tacrolimus, mycophenolic acid and corticosteroids) and by low molecular weight heparin. Anti-PF4/H Ab were detected using the ELISA Asserachrom HPIA (Diagnostica Stago, France). For positive (POS) sample, the Ig isotype was determined (Asserachrom HPIA - IgG, Diagnostica Stago).

Results: PF4/H Ab were observed in 30.5% of the patients on the T2 sample. No patients (32 tested, among them 12 were POS on T1 sample) had PF4/H Ab 3 on the T3 sample. Among the 18 POS plasma, 11 were of IgG isotype. There was no association of PF4/H Ab with the type of lung pathology or the use or the duration of extracorporeal membrane oxygenation (see Table). Inflammatory state, evaluated by leukocyte and fibrinogen levels was not different in patients with or without PF4/H Ab. The presence of PF4/H Ab was not associated with the appearance of a thrombotic event during a 3 month follow-up and no patient with PF4/H Ab developed thrombocytopenia.

Table

	Absence of PF4/H Ab (n =41)	Presence of PF4/H Ab (n =18)	P (Absence vs. presence)
CF/COPD (n)	23/18	12/6	0.18
ECMO post-op (n / median duration in days)	(11/5)	(4/5.5)	(0.95/0.66)
Thrombotic event (n)	24	7	0.56
Deceases during follow-up (n)	5	1	0.75
Leukocytes (G/L) (T1/T2)	10.7/13.2	11.9/11.5	0.27/0.27
Platelets (G/L) (T1/T2)	286/381	281/449	0.99/0.16
Fibrinogen (g/L) (T1/T2)	4.03/5.1	4.05/6.1	1/0.13

Conclusions: Despite a strong immunosuppressive regimen, a transient high frequency of PF4/H Ab is observed in patients undergoing BPT, indicating that T cells likely have a limited role in the immune response to PF4/H complexes. PF4/H Ab is not a marker of the thrombotic risk in BPT.

PLA27

Clinical significance of low $CD4^+/CD8^+$ ratio in patients with immune thrombocytopenic purpura: A single centre study

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Background: Immune thrombocytopenic purpura (ITP) is an autoimmune disorder. It is associated with a loss of tolerance to platelet antigens and a phenotype of accelerated platelet destruction and impaired platelet production. The T-cells are considered to play an important role in the pathogenesis of ITP.

Aims: To evaluate the imbalance in helper and cytotoxic T cells ratio in ITP.

Methods: The study was performed at National Institute of Blood Diseases & Bone Marrow Transplantation, Karachi from January to December, 2015. Total 50 ITP patients and 30 healthy volunteers were included in this study. Complete blood count was performed on automated hemo-analyzer XN-1000 and morphological examination of blood was done in duplicate by two different microscopists. The $CD4^+/CD8^+$ ratio was determined by BD Tri-testCD4 FITC/ CD8 PE/ CD3 PerCP (Cat #: 340298) antibody cocktail using BD FACS Calibur flowcytometer. About 50µl of whole blood was taken in Trucount tube and 20µl of antibody cocktail was added. The tube was incubated at 25ºC and standard protocol by BD was followed to fix the cells. The data was acquired and analyzed by using Multiset version 3.0.

Results: Out of total 50 patients, 21(42%) were males and 29(58%) were females. The mean age of the patients was 35 years (range: 3-80 years). The mean platelet count was $91.7x10^3/\mu l$. Platelet anisocytosis with decreased platelet count was observed in 35 (70%) patients. The mean CD4/CD8 ratio was 0.8 compared to controls 1.09.

Conclusions: In ITP, T-cell plays an important role in pathogenesis. The ratio of CD4/CD8 measures the balanced of immune system reflecting health. In ITP, decreased platelet count and low CD4/CD8 ratio are observed which is useful in determining treatment response and disease progression. More studies are needed for T-cell involvement in pathogenesis of ITP.

PLA28

An activating mutation of *DIAPH1* encoding rho-effector diaphanous-related formin-1 causes inherited macrothrombocytopenia associated with sensorineural hearing loss in a french family

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Background: Variants of many genes cause inherited macrothrombocytopenia (MTP) associated or not with syndromic disorders. Nevertheless for many affected families the genetic defect is unknown.

Aims: To identify the gene variant responsible for a familial MTP associated with hearing loss but normal *MYH9*, moderate bleeding, mild neutropenia and normal platelet aggregation.
Methods: Whole exome sequencing, performed in the course of the European BRIDGE project for 120 French families, identified a candidate gene variant selected on the basis of genetic, clinical and biological characteristics. Co-segregation was performed. Evaluation of platelet morphology, cytoskeletal structures, megakaryocytopoiesis and heterologous cell culture experiments completed the study.
Results: A heterozygous premature stop codon, candidate variant *DIAPH1* R1213* co-segregated in 5 members of a family with giant platelets and hearing loss. DIAPH1 is a Rho GTPase effector involved in F-actin assembly and microtubule dynamics. The patients' platelets possess normal levels of GPIb, filamin and myosin-IIA, while residual DIAPH1 was present, but abnormally sensitive to proteolysis. Platelets were characterized by an increase in F-actin and an elevated presence of stable microtubules as analyzed by EM, IF, and WB. Overexpression of DIAPH1 R1213* in heterologous cells reproduced the cytoskeletal alterations. CD34+ cell-derived megakaryocyte cultures confirmed a defect of proplatelet production. The R1213* stop codon variant is located in a crucial auto-regulatory domain of DIAPH1 that is normally released after binding of activated Rho GTPases. Overall, our results are in favor of a gain of function variant of DIAPH1.
Conclusions: In the absence of the auto-inhibitory motif, platelets contain a constitutively active DIAPH1 that disrupts F-actin organization and increases the stability of microtubules. Our results identify a mutation in a Rho family effector and help to define a novel form of MTP associated with hearing loss.

PLA29

Increased platelet expression in HIV positive treatment naive patients does not correlate with CD4 count

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Background: HIV /AIDS despite causing thrombocytopenia is paradoxically associated with an increased incidence of clinical thrombosis whose mechanism is still unsettled. This study investigated P-selectin expression in treatment naïve HIV patients in the absence of agonist stimulation.
Aims: to determine p-selectin expression in treatment naive HIV positive symptomatic patients.
Methods: Following ethical approval blood was collected from 15 HIV positive treatment naïve and 11 HIV negative subjects (controls) in citrated tubes after which platelet P-Selectin expression was immediately analyzed using FACS CALIBUR (BD) using Cellquest software. Immunolabeling was by FITC CD62P. Statistical analysis was by Mann-Whitney U Test using SPSS 16.0 and significance set $P < 0.05$.
Results: Median Platelet % P-Selectin expression levels in HIV positive group was 1.5 times the control group (34.5 (IQ) 10.3-63.3 vs 21.1 (IQ) 2.7-46.2). There was no correlation between the p-selectin expression and CD4 count.
Conclusions: This study demonstrates an increased platelet activation in HIV positive patients. The lack of correlation of P-selectin expression with the presence of increased viral load indicates that CD4 count is a less sensitive predictive marker of platelet reactivity. Future should investigate the platelet reactivity levels in clinical thrombotic events in HIV patients.

Platelet Physiology

PP01

Outward migration of procoagulant platelets to thrombus surface is driven by thrombus contraction and decreased adhesivity

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Background: Procoagulant platelets are highly activated subpopulation with high level of phosphatidylserine externalization, decreased adhesivity, balloon-like shape and other specific traits. Their (patho)physiological role continues to be debated.
Aims: To get insight into dynamics of procoagulant platelets formation and their impact on thrombus architecture we investigated fates of individual procoagulant platelets during thrombosis.
Methods: Real-time imaging with confocal and epifluorescence microscopes was used for analysis of thrombus formation on collagen under microvascular flow conditions in a flow chamber system. A computational model for studying dynamics of non-adhesive spheres within contracting thrombus was developed.
Results: Real-time microscopy showed that procoagulant platelets formed inside a thrombus moved towards its surface. This resulted in a ring-like distribution of procoagulant platelets around the base of the thrombi near collagen. We assumed that this motion was driven by thrombus contraction along with reduced adhesivity of procoagulant platelets. This hypothesis was in line with results obtained using blood of MYH9 mice (with deficient nonmuscle myosin and, therefore, no thrombus contraction): for knockouts, procoagulant platelets were distributed within the thrombus and did not migrate to its outer surface. Computational modeling demonstrated that mechanical contraction of thrombus consisting of adhesive spheres leads to mechanical extrusion of non-adhesive spheres to its surface.
Analysis of primary fibrin generation sites distribution yielded some correlation with procoagulant platelets localization: in WT mice fibrin originated at the surface of thrombi, while for MYH9 mice fibrin generation sites were distributed throughout the thrombi.
Conclusions: Procoagulant platelets are mechanically expelled to thrombus surface by thrombus contraction process. Surface distribution of procoagulant platelets might be responsible for observed spatial features of fibrin generation.

PP02

Defects in TRPM7 channel function deregulate thrombopoiesis through altered cellular Mg^{2+} homeostasis and cytoskeletal architecture

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Background: Mg^{2+} is the second most abundant divalent cation in mammalian cells and plays a decisive role in platelet function. Despite implications for life-threatening conditions such as stroke or myocardial infarction, the mechanisms controlling $[Mg^{2+}]_i$ in megakaryocytes (MK) and platelets are entirely unknown. Transient receptor potential melastatin-like 7 channel (TRPM7) is a ubiquitously expressed, constitutively active Mg^{2+} and Ca^{2+} permeable ion channel covalently fused to a cytosolic α-type serine/threonine protein kinase domain, which is critical for cell survival. Although TRPM7-mediated cation influx has been detected in MKs, its role in thrombopoiesis has not been investigated to date.

Aims: We investigated the role of TRPM7 in MK cation homeostasis and thrombopoiesis.

Methods: We analyzed MKs and platelets of conditional TRPM7-deficient mice (*Trpm7$^{fl/fl\text{-}Pf4Cre}$*) as well as from a human pedigree with variants in the TRPM7 channel domain using a wide range of *in vitro* and *in vivo* methods.

Results: We report that impaired channel function of TRPM7 in MKs causes macrothrombocytopenia in *Trpm7$^{fl/fl\text{-}Pf4Cre}$* mice and in several members of a human pedigree also featuring atrial fibrillation. The defect in platelet biogenesis was mainly caused by cytoskeletal alterations resulting in impaired proplatelet formation by *Trpm7$^{fl/fl\text{-}Pf4Cre}$* MKs, which was rescued by Mg^{2+} supplementation or chemical inhibition of non-muscle myosin IIA heavy chain (*MYH9*, NMMIIA) activity. Strikingly, platelets from patients with impaired TRPM7 channel function recapitulated the aberrant cytoskeletal architecture found in platelets from *Trpm7$^{fl/fl\text{-}Pf4Cre}$* mice.

Conclusions: Collectively, our findings reveal TRPM7 dysfunction as a novel cause of macrothrombocytopenia in humans and mice and suggest dietary Mg^{2+} supplementation as a potential treatment of patients with increased activity of NMMIIA to manage thrombocytopenia.

PP03

Clinical and pathogenetic characterization of *ETV6*-related thrombocytopenia (*ETV6*-RT), an inherited thrombocytopenia (IT) predisposing to childhood acute lymphoblastic leukemia (ALL)

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Background: *ETV6*-RT is an autosomal dominant IT recently identified in a few families and suspected to predispose to hematological malignancies.

Aims: To gain information on the clinical and laboratory picture of this new IT, in particular on predisposition to hematological malignancies.

Methods: 130 unrelated and consecutive patients with ITs were enrolled: all of them had no definite diagnosis because they did not fit the criteria for any known IT. *ETV6* mutations were investigated by WES or Sanger sequencing: whenever *ETV6* mutations were identified, all available relatives of probands were also investigated. 5 patients from 2 known *ETV6*-RT families already partially described (Noetzli L et al, Nat Genet 2015, 47:535) were also included.

Results: Overall, 20 subjects from 7 families bearing 5 different *ETV6* mutations were identified. The bleeding tendency and the degree of thrombocytopenia were mild, but 4 patients from 3 families had childhood B-cell ALL, confirming that *ETV6*-RT is an ALL predisposition syndrome. Clinical and laboratory findings did not identify any peculiar defect that can be used to suspect this disorder. *In vitro* studies revealed that patient megakaryocytes have defective maturation and proplatelet formation, while platelets have reduced ability to spread on fibrinogen. At variance with most ITs, platelet size was not enlarged: this finding is shared with ITs due to monoallelic mutations in *RUNX1* and *ANKRD26*, which also have normal platelet size and predispose to leukemia.

Conclusions: Our study showed that monoallelic *ETV6* mutations cause one of the most frequent forms of ITs and confirmed that affected subjects have little bleeding tendency but high propensity to hematological malignancies, in particular childhood ALL. Since *ETV6*-RT is one of the few autosomal dominant forms of IT without platelet macrocytosis, the screening for *ETV6* mutations is recommended in all patients with these characteristics, along with the screening for *RUNX1* and *ANKRD26* mutations.

PP04

PDK1 determines collagen-dependent platelet activation and is critical to development of ischemic stroke *in vivo*

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Background: Platelet activation by subendothelial collagen results in an increase of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) and is followed by platelet secretion, aggregation and arterial thrombus formation.
Aims: The present study aimed to determine the role of 3-phosphoinositide-dependent protein kinase-1 (PDK1) in collagen-dependent platelet activation, arterial thrombosis and ischemic stroke *in vivo* in a platelet-specific knockout approach.
Methods: FACS, immunoblotting, luminescence aggregometry, DIC, ELISA, spectrofluorimetry, flow chamber, $FeCl_3$-induced vascular injury, tMCAO, MRI.
Results: Platelet activation with collagen receptor GPVI agonists CRP or convulxin resulted in an activation of PDK1. PDK1-deficient platelets ($pdk1^{fl/fl}pf4^{Cre/+}$) displayed a strongly blunted platelet phospholipase Cγ2-dependent inositol triphosphate production and severely defective increase of $[Ca^{2+}]_i$ in response to GPVI agonists as compared to platelets from wildtype littermates ($pdk1^{fl/fl}pf4^{+/+}$). Impaired increase of $[Ca^{2+}]_i$ resulted in a substantial defect in activation-dependent platelet secretion and aggregation in $pdk1^{fl/fl}pf4^{Cre/+}$ platelets upon stimulation with CRP as compared to $pdk1^{fl/fl}pf4^{+/+}$ platelets. PDK1-deficient platelets displayed a significantly diminished Rac-1 activation, which was paralleled by abrogated lamellipodia formation and spreading on fibrinogen after stimulation with CRP. Adhesion to collagen and thrombus formation under high arterial shear rates were significantly abolished in $pdk1^{fl/fl}pf4^{Cre/+}$ platelets. $Pdk1^{fl/fl}pf4^{Cre/+}$ mice were protected against arterial thrombotic occlusion after $FeCl_3$-induced mesenteric arterioles injury and ischemic stroke *in vivo*. $Pdk1^{fl/fl}pf4^{Cre/+}$ mice showed significantly reduced brain infarct volumes with a significantly increased survival 7 d after tMCAO without increase of intracerebral hemorrhage.
Conclusions: PDK1 is required for Ca^{2+}-dependent platelet activation upon stimulation of GPVI, arterial thombotic occlusion and ischemic stroke *in vivo*.

PP05

Three-dimensional electron microscopy reveals new details for platelet granule exocytosis: granule-to-granule vs. granule-to-platelet membrane fusion

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Background: Granule secretion is pivotal in many platelet responses. The fusion routes by which the contents of α and δ granules are released remain uncertain.
Aims: We visualize the spatial and temporal organization of granules in activated platelets.
Methods: by using a 3D reconstruction approach based on electron microscopy.
Results: Two distinct modes of exocytosis, single and compound exocytosis, were characterized depending on the concentration of thrombin used to stimulate platelet. Weak stimulation favors single exocytosis, i.e. the fusion of individual granules with the platelet membrane. Higher levels of stimulation induce compound exocytosis, i.e granule-to-granule fusion, resulting in the formation of large multigranular compartments which is followed by plasma membrane fusion. While α granules use both modes of exocytosis, δ granules go only through single exocytosis. To define the underlying molecular mechanisms, we examined platelets from Vesicle-Associated Membrane Protein (VAMP)-8 null mice. We found that α-to-α granule fusion were abolished in platelet from VAMP-$8^{-/-}$ mice, while fusion of individual granules was still observed, indicating that VAMP-8 is important in platelet compound exocytosis. The size of the fusion pores varies, with the initial pores being relatively small (~40 nm) and the later ones larger (~150 nm). This may regulate the release of different cargo molecules depending on their size. The biological relevance of both modes of exocytosis, was addressed by analyzing granules in *in vivo* formed thrombi. Compound exocytosis was observed in thrombi produced after severe laser injuries where thrombin is generated. Under conditions of lower injury, so as for the superficial lesion, no multigranular components were detected.
Conclusions: Altogether, this 3D study shows that platelets possess two distinct modes of exocytosis which may be relevant in the regulation of multiple platelet functions.

PP06

Blood coagulation factors bound to procoagulant platelets are concentrated in their cap structures to promote clotting

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Background: Binding of coagulation factors to phosphatidylserine (PS)-exposing procoagulant activated platelets and formation of membrane-dependent complexes on their surface are critical for blood coagulation. Procoagulant platelets have a special morphology including a small (approximately 1 μm radius) "cap"-like convex region containing a lot of adhesive proteins, and a large "balloon" structure.
Aims: The aims of this study was characterised of spatial distribution of coagulant factors on the membrane of activated platelets.
Methods: The spatial distribution of coagulation factors to activated platelets was studied with an Axio Observer Z1 confocal microscope (Carl Zeiss, Jena, Germany) using fluorescein-labeled coagulation factors or specific antibodys. For study structure of the "caps" was used transmission electron microscope JEM-1400 (JEOL Tokyo, Japan).
Results: Here we show that blood coagulation factors bound to procoagulant platelets including factors IXa (FIXa), Xa/X (FXa/FX), Va (FVa), VIII (FVIII), prothrombin, and phosphatidylserine-sensitive marker annexin V are co-localized and highly concentrated in the "caps" of procoagulant platelets. A three-dimensional computer simulation model of intrinsic tenase based on these data revealed how localization of proteins in the cap can promote procoagulant reactions by two orders of magnitude due to increased local concentrations. Transmission electron microscopy indicated a complex structure of the

"caps" with numerous membrane folds possibly suggesting additional roles of this phenomenon. In platelet thrombi formed in whole blood on collagen under arterial shear conditions, ubiquitous "caps" with increased annexin V binding were observed indicating relevance of this mechanism under physiological conditions.
Conclusions: In this work was shown an essential heterogeneity in the surface distribution of major coagulation factors on the surface of procoagulant platelets and suggest its importance in promoting membrane-dependent coagulation reactions.

PP07

Heat shock protein 70 (Hsp70) regulates platelet integrin activation, granule secretion and aggregation

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Background: Molecular chaperones that support protein quality control, including heat shock protein 70 (Hsp70), participate in diverse aspects of cellular and physiological function. Recent studies have reported roles for specific chaperone activities in blood platelets in maintaining hemostasis; however, the functions of Hsp70 in platelet physiology remain uninvestigated.
Aims: In the present study, we aimed to characterize roles for Hsp70 activity in platelet activation and function.
Methods: *In vitro* biochemical, microscopy, flow cytometry and aggregometry assays of platelet function as well as *ex vivo* analyses of platelet aggregate formation in whole blood under shear were carried out under Hsp70-inhibited conditions.
Results: Inhibition of platelet Hsp70 blocked platelet aggregation and granule secretion in response to collagen-related peptide (CRP), which engages the ITAM-bearing collagen receptor GPVI/FcRγ complex. Hsp70 inhibition also reduced platelet $\alpha_{IIb}\beta_3$ activation downstream of GPVI, as Hsp70-inhibited platelets showed reduced PAC-1 and fibrinogen binding. *Ex vivo*, pharmacological inhibition of Hsp70 in whole human blood prevented the formation of platelet aggregates on collagen under shear. Biochemical studies supported a role for Hsp70 in maintaining the assembly of the LAT signalosome, which couples GPVI-initiated signaling to integrin activation, secretion and platelet function.
Conclusions: Together, our results suggest that Hsp70 regulates platelet activation and function by supporting LAT-associated signaling events downstream of platelet GPVI engagement, suggesting a role for Hsp70 in the intracellular organization of signaling systems that mediate platelet secretion, "inside-out" activation of platelet integrin $\alpha_{IIb}\beta_3$, platelet-platelet aggregation, and ultimately hemostatic plug and thrombus formation.

PP08

Severe aortic stenosis-induced platelet activation and high plasma lipoxygenase products are rapidly corrected by transcatheter aortic valve replacement

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Background: The consequences of repeated exposure of platelets to high shear stress, as created by severe aortic valve stenosis (AS), are hardly predictable. Changes in platelet activation and reactivity before, during, and after Transcatheter Aortic Valve Replacement (TAVR) have never been studied.
Aims: To investigate platelet function in patients with severe AS and its evolution within the first week after TAVR.
Methods: 18 high-risk patients undergoing trans-femoral TAVR (Medtronic Corevalve) were prospectively enrolled after giving informed consent. Device success was achieved in all. Various membrane or soluble markers of in vivo platelet activation, circulating platelet/leukocyte aggregates, plasma lipidomic analysis and in vitro platelet reactivity to various agonists were assessed in peripheral venous blood before (S0), 1 (S1) and 5±1 (S5) days after TAVR. Plasma serotonin and 12-LOX products were measured in blood sampled in ascending aorta per-procedure, before and within min after valve deployment. 26 atherosclerotic patients without AS formed a control group. TAVR and control patients were treated by low dose aspirin monotherapy.
Results: Before TAVI, in vitro responsiveness (aggregation and secretion) were in the low normal range, without difference between AS and controls. In vivo platelet activation (sGpVI, and sCD40L release) was noticed in AS, but the most robust and sensitive markers were a high level of platelet/monocytes aggregates and plasma 12-LOX products (12-HETE and 14-HDoHE). On day 1 after TAVI, these markers dropped to the levels of controls (see Figure). In blood sampled in ascending aorta per-procedure, we did not detect acute procedure-related platelet activation on the bioprosthesis.

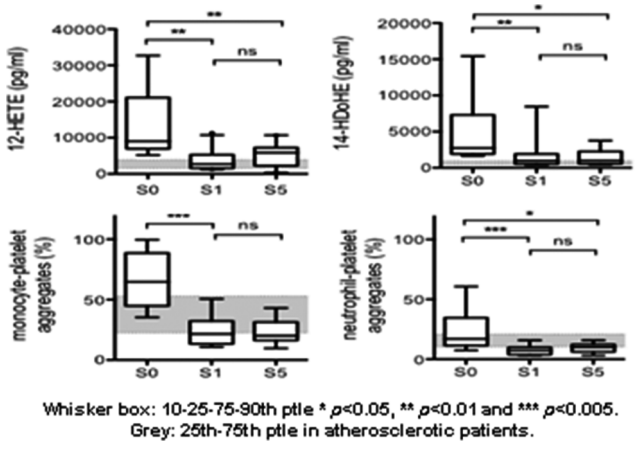

Figure Correction of platelet activation by TAVR.

Conclusions: Strong in vivo platelet activation is associated to severe AS, the consequences of which, on long term, for progression of valve sclerosis and systemic atherosclerosis are likely. This is rapidly corrected after successful TAVR, demonstrating the role of pathological shear stress in this process.

PP09

Risk factors for clopidogrel high on-treatment platelet reactivity in patients with carotid artery stenosis undergoing endarterectomy

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Background: Carotid endarterectomy (CEA) is the standard treatment for carotid stenosis. Dual antiplatelet therapy, including aspirin and clopidogrel, has a potential role in reducing the risk of stroke after carotid surgery. However, clopidogrel high on-treatment platelet reactivity (HTPR) is quite a common phenomenon.
Aims: Our study aimed to evaluate genetic and non-genetic factors as possible risks for clopidogrel-HTPR in patients with carotid artery stenosis undergoing CEA.
Methods: Using multiple-electrode impedance aggregometry (MEA) the antiplatelet effectiveness of clopidogrel was prospectively evaluated in 112 patients (66.2 ± 8.1 years). Measurements were made after 24 h, 7 and 30 days of clopidogrel treatment, which was introduced after elective CEA at a dose of 75 mg daily, for at least 30 days. Clopidogrel-HTPR was defined as an adenosine diphosphate (ADP) - thrombin receptor activating peptide (TRAP) platelet aggregation ratio ≥ 52%. *CYP2C19*2* genotyping was performed by TaqMan assay. Logistic regression models were used to estimate predictors for low responsiveness. The Ethics Committee of the "Dedinje" Institute for Cardiovascular Diseases approved the research protocol. All patients gave written informed consent prior to study inclusion.
Results: According to this specific cut-off value for our population, the number of patients with HTPR declined from 79.5% 24 h after introducing clopidogrel to 25% after 30 days of treatment. Analysis showed that 16/30 patients carrying the *CYP2C19*2* gene variant had HTPR, in contrast to 12/82 non-carriers of this allele ($P < 0.001$). Multivariate logistic regression analysis identified the *CYP2C19*2* gene variant (OR 4.384, 95% CI 1.296-14.833, $P=0.017$) and high total cholesterol (OR 2.090, 95% CI 1.263-3.459, P=0.004) as the only independent risk factors for clopidogrel-HTPR.
Conclusions: The *CYP2C19*2* gene variant and high total cholesterol level were risk factors for clopidogrel-HTPR in patients with carotid artery stenosis undergoing CEA.

PP10

Defining the Mac-1 I-domain binding site of the GPIbα N-terminal domain

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Background: The interaction between leukocyte integrin Mac-1 and platelet GPIbα is critical in the inflammatory response to vascular injury. Within the Mac-1 heterodimer the I-domain has been identified as the site of this interaction.
Aims: To define the key residues involved in the Mac-1 binding interface with GPIbα, through the NMR characterization of the I-domain and consequently to determine the importance of the MIDAS for binding.
Methods: Murine GPIbα N-terminal domain was expressed using *Drosophilia*. The secreted protein was captured from media using Ni-affinity and purified by gel filtration and ion exchange. Murine Mac-1 I-domain was expressed in *E.coli*. For NMR purposes M9 minimal media was used, supplemented with $^{15}N/^{13}C$ as required. The protein was obtained from the soluble fraction of the cell lysate and purified using GST-affinity and ion exchange. NMR experiments were recorded at 25°C on a Bruker 800 MHz Avance III spectrometer. GPIbα N-terminal domain was titrated into a ^{15}N-enriched sample of I-domain to a 2:1 excess. Chemical shift perturbation was quantified as: $CSP = \sqrt{½}(\Delta H^2 + 0.14 * \Delta N^2)$, perturbation was deemed significant by the threshold > μ+σ. SPR was performed using a BIAcore 3000. GPIbα N-terminal was amine-coupled to a CM5 sensor chip and I-domain samples ranging from 11 to 200 μM (1 mM $MgCl_2$) were injected at a flowrate of 50 μL/min. Regeneration was achieved using 2 M NaCl.
Results: CSP NMR analysis of I-domain residue signals perturbed during GPIbα titration reveals the location of the binding site. SPR sensograms show an increase in affinity for the F302W mutant in comparison to the wild-type, whereas the T209A mutant results in a total loss of binding. A similar effect was seen for all constructs when EDTA was added.
Conclusions: The key residues involved in the interaction between Mac-1 I-domain MIDAS and GPIbα N-terminal domain have been identified. SPR studies provide further evidence of the metal-dependence and structural-dependence to binding affinity.

PP11

Platelets are dispensable for antibody-mediated transfusion-related acute lung injury in the mouse

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Background: Transfusion-related acute lung injury (TRALI) is a serious transfusion-related complication. Previous conflicting studies indicated that platelets are either crucial or dispensable for TRALI.
Aims: To evaluate the role of platelets in MHC I-induced-TRALI.
Methods: Antibody-mediated TRALI was experimentally induced in mice by lipopolysaccharide priming followed by the administration of an anti-MHC I monoclonal antibody (mAb).
Results: TRALI was tested in the context of severe thrombocytopenia provoked by the administration of diphtheria toxin (DT) in transgenic iDTR mice selectively expressing DT receptor in megakaryocytes. The pathological responses occurring within the first 10 min following the injection of the anti-MHC I mAb, i.e., the severity of lung edema and the drop in aortic blood oxygenation, were similar in severely thrombocytopenic DT-iDTR and control mice. At later times, mortality was nevertheless increased in DT-iDTR mice, due to lung hemorrhages. When less severe thrombocytopenia was induced with an anti-platelet mAb, TRALI initiated and developed similarly as in control mice, but hemorrhages were absent. Furthermore, when platelet functions were defective due to administration of aspirin or clopidogrel or to glycoprotein GPIIbIIIa deficiency, TRALI still developed while no lung hemorrhages were observed. In contrast, when GPVI was immunodepleted, TRALI still occurred but was occasionally accompanied by hemorrhages.

Conclusions: Platelets are dispensable for the initiation and development of MHC I-induced TRALI. Although they do not protect against the disruption of vascular endothelial cell barrier and the subsequent plasma leakage and edema formation, platelets are however essential to prevent more serious damage resulting in hemorrhages in alveoli.

PP12

Procoagulant platelets and the role of mitochondria in their formation

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Background: Platelet activation leads to the formation of two distinct subpopulations: one of them consists of amoeboid well-aggregating platelets with operating mitochondria and no phosphatidylserine (PS) on their surface, while another (called "procoagulant") includes balloon-like cells with externalized PS, depolarized mitochondria and a cap of alpha-granule proteins, but without ability for aggregate formation. It is established that the subpopulations are not pre-existing: the fraction of procoagulant platelets increases with the degree of activation, and can be changed between 0 and 90%. Signal transduction mechanisms that define formation of these subpopulations are presently unclear.
Aims: Revealing relationship between calcium dynamics and mitochondrial collapse in procoagulant platelet formation.
Methods: Signal transduction in platelets during activation was measured with real-time confocal microscopy of single fibrinogen-bound platelets loaded with fluorescent dyes sensitive for calcium in cytosol, mitochondria and mitochondrial membrane potential in presence of the PS marker Annexin V.
Results: Stimulation of platelets using thrombin or PAR1 agonist SFLRRN initially produces a series of stochastic cytosolic calcium spikes. Some platelets remain in this state. In others, there is a transition from spikes to the sustained high calcium, that could be the result of the uptake of cytosolic calcium spikes by mitochondria leading to its overloading, mPTP opening, and PS externalization.
Conclusions: These results support the model of procoagulant subpopulation development following a series of stochastic cytosolic calcium spikes that are accumulated by mitochondria leading to the collapse, and suggest important roles of individual platelet reactivity and signal exchange between different mitochondria of a single platelet. Higher cytosolic calcium in a resting platelet increases its chances of death after activation.

PP13

Myr-RK6 peptide selectively regulates outside-in signaling transduction-related functions in human platelets

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Background: Integrin αIIbβ3 serves as the common pathway for bidirectional signaling during platelet activation and aggregation, in which talin plays a pivotal role.
Aims: To assess the effect of the interaction of the talin rod domain integrin binding site 2 with integrin β3 on platelet signal transduction.
Methods: we designed and synthesized a peptide that mimics the membrane proximal α helix 6 residues $R^{724}KEFAK^{729}$ of the integrin β3 cytoplasmic tails, to which the myristoylation was covalently linked to the N-terminal of the peptide enabling membrane penetration. The effects of myr-RKEFAK peptide on the typical platelet outside-in (stable adhesion and spreading on immobilized fibrinogen, aggregation, fibrin clot retraction) and inside-out signaling events
(soluble fibrinogen binding) were tested.
Results: The results showed that myr-RK6 peptide dose-dependently inhibited platelet stable adhesion and spreading on immobilized fibrinogen, irreversible aggregation, as well as fibrin clot retraction, but not soluble fibrinogen binding and reversible phase of platelet aggregation.
Conclusions: It is concluded that cell-penetrating peptide myr-RK6 caused an inhibitory effect on integrin β3 outside-in signaling-regulated platelets functions, but did not affect inside-out signaling-regulated platelets functions.

PP14

Regulation of αIIbβ3 signalling by the ITIM-containing receptor G6b-B

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Background: G6b-B is an immunoreceptor tyrosine-based inhibition motif (ITIM)-containing receptor that negatively regulates signalling from the immunoreceptor tyrosine-based activation motif (ITAM)-containing collagen receptor complex GPVI-FcR γ-chain and the hemi-ITAM-containing podoplanin receptor CLEC-2. However, mice lacking G6b-B exhibit defects in platelet count and function that cannot be fully explained by increased GPVI-FcR γ-chain and CLEC-2 signalling, suggesting G6b-B has additional functions.
Aims: To determine the role of G6b-B in regulating integrin αIIbβ3-mediated signalling and function.
Methods: Tyrosine phosphorylation of G6b-B and interaction with the non-transmembrane protein-tyrosine phosphatases (PTPs) Shp1 and Shp2 was analysed in resting and activated human and mouse platelets by immunoprecipitation and western blotting. Compartmentalization of G6b-B, Shp1 and Shp2 in lipid and non-lipid rafts was investigated by sucrose gradient ultra-centrifugation. Platelets from G6b-B-deficient mice were analysed for defects in αIIbβ3 signalling and functional responses.
Results: Tyrosine phosphorylation of G6b-B and association with Shp1 and Shp2 increased dramatically in aggregated platelets, activated either by collagen-related peptide (CRP) or thrombin, and in fibrinogen-adhered platelets. G6b-B, Shp1 and Shp2 were almost exclusively localised to non-lipid rafts in resting and CRP-activated platelets. Thrombin-pre-activated platelets from G6b-B-deficient mice exhibited reduced spreading on fibrinogen. Whole cell tyrosine phosphorylation was altered in fibrinogen-adhered G6b-B-deficient platelets, with some bands being hyper-phosphorylated, whereas others were hypo-phosphorylated.
Conclusions: Findings from this study demonstrate that G6b-B is phosphorylated downstream of αIIbβ3 and forms a signalling complex with Shp1 and Shp2 that subsequently regulations outside-in signalling from αIIbβ3.
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PP15

ABO(H) blood group expression modifies platelet function

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Background: The platelet glycoprotein (GP)Ib and von Willebrand Factor (VWF) both express covalently linked ABO(H) blood group carbohydrate determinants. Blood group O is associated with an increased risk of bleeding whereas non-O is associated with increased risk of myocardial infarction. It is not clear why.
Aims: We set out to characterize platelet function from donors with blood group O and non-O.
Methods: Platelet function was assessed in response to shear in platelet rich plasma (PRP) at 120dyn using a cone and plate viscometer. P-Selectin expression levels, annexin V binding and a count of disappearing platelets were used to determine functional responses to shear. Platelet aggregation in response to incremental concentrations of Arachidonic Acid, Collagen, ADP, Epinephrine, Ristocetin and TRAP-6 was assessed using a 96-well plate assay. A parallel plate perfusion chamber was used to measure platelet interaction with VWF at arterial shear (1500^{s-1}).
Results: There were no between group (10 Type O vs. 10 non-O) differences in response to shear. In aggregation studies, the dose response curve to Ristocetin (0.2mg/ml - 1.5mg/ml, $p \leq 0.004$, non-linear fit) and TRAP-6 (0.16μM - 20μM, $p \leq 0.003$) was significantly lower in platelets from donors with Type O blood (n=17) compared to non-O donors (n=17,). Under conditions of arterial flow platelets from Type O donors (n=32) travelled greater distances over pooled VWF than those from non-O donors (n=54) (9.2±3.3 vs 7.5±3.2μM, mean±SD, $p \leq 0.004$, Mann-Whitney t-test). Platelet velocity for Type O donors was significantly higher (4.6±2.5 vs 3.4±2.4μM/sec, $p \leq 0.006$).
Conclusions: The results of this investigation demonstrate that platelets from donors with Blood Type O are less reactive than non-O platelets. Our aggregation and shear flow experiments suggest that this lower reactivity is mediated through the specific interaction of the platelet GPIb receptor and its ligand, VWF.

PP16

Fibrin fibers from cardiovascular disease patients on aspirin are more extensible than fibers from healthy individuals

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Background: In past research, we established that single fibrin fibers are extraordinarily extensible and elastic, and that they have a stretch modulus on the order of a few MPa. These data were taken on purified fibrinogen. These single fibrin fiber mechanical properties are likely important for fibrin function.
Aims: Our aim is to determine if single fibrin fiber mechanical properties are altered in cardiovascular disease patients who took low or medium dose aspirin.
Methods: We used a combined atomic force/fluorescence microscopy technique to determine the mechanical properties of single fibrin fibers formed from the plasma of the following groups.
Blood samples were taken from five males in three different groups. Healthy younger (< 50 yrs.) and healthy older males (>70 yrs.), and older males with cardiovascular disease (CVD) who took low or medium dose aspirin.
Results: 1) Age did not have an effect on the stretch modulus (stiffness), extensibility or elasticity. 2) Fibrin fibers from older males with CVD who took aspirin were 30% more stretchable and 50% more elastic than those from the healthy control groups. 3) The modulus, Y, in all fibers strong decreases with increasing fiber radius, R. For all healthy individuals the modulus varied as $Y \sim R^{-1.5}$, whereas it varied as $Y \sim R^{-1.0}$ for individuals from the CVD + aspirin group.
Conclusions: The strong decrease of the modulus with increasing radius is consistent with a new fiber model, in which fibers have a denser core and a less dense periphery. Cardiovascular disease and/or aspirin use affect the mechanical properties and internal structure of single fibrin fibers.

PP17

The impact of *CYP2C19* *2, *3, *CYP4F2* *3, *Fibrinogen β* (C-148T) genotype and clinical factors on antiplatelet effect during induction of ticagrelor and aspirin therapy

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Background: Ticagrelor is recommended over clopidogrel in patients following percutaneous coronary intervention. Still no SNP's were identified to impact ticagrelors' antiplatelet effect.
Aims: The aim of the study was to reveal whether clinical factors, *CYP2C19*, *CYP4F2* and *fibrinogen* genotype have a significant effect on platelet reactivity during induction of ticagrelor and aspirin therapy.
Methods: Totally 86 patients, who had been hospitalized due to acute coronary syndromes at the Department of Cardiology, Lithuanian University of Health Sciences (LUHS), from 09 of 2015 to 01 of 2016 and who followed dual ticagrelor and aspirin therapy for at least 48 h after stent implantation were included into the further study. A standard 90 mg twice per day ticagrelor and 100 mg/day aspirin doses were prescribed to all of the represented patients. The polymorphisms of *CYP2C19* *2 (rs4244285), *3 (rs4986893), *CYP4F2* G1347A (*rs2108622*) and *fibrinogen* C-148T (rs1800787) were assessed in the laboratory of Molecular Cardiology of the Institute of Cardiology of LUHS. This study was done according to the Declaration of Helsinki. Written informed consent was obtained from all patients included in the study.
Results: Platelet aggregation after induction with ADP (PA-ADP) was higher in females (n=21) 33.14±12.97 $\%^{agr}$ than in males 25.51±11.65 $\%^{agr}$, p=0.01. Also, patients with diabetes (n=24) had higher PA-ADP levels 32.13±15.57 $\%^{agr}$ than non-diabetic patients 25.53±10.43$\%^{agr}$, p=0.02. Smoking or proton pump inhibitor use, *CYP2C19* and *CYP4F2* alleles had no effect on PA-ADP levels. PA-ADP platelet aggregation was significantly higher in fibrinogen CC genotype carriers (n=17) 36.88±13.69 $\%^{agr}$ compared to CT (n=26) 24.42±11.77 $\%^{agr}$, p=0.002, or TT (n=43) carriers 25.40±10.43 $\%^{agr}$, p=0.0009.
Conclusions: The results of this study revealed that platelet aggregation during induction of antiplatelet therapy with ticagrelor and aspirin was impacted by patient gender, diabetes and fibrinogen genotype.

PP18

Development and upscaling of the purification process of snake venom antiplatelet agents

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Background: A wide range of studies on snake venom-derived proteins with platelet inhibitory properties have provided new opportunities in diagnosis and treatment. Nevertheless, more antiplatelet enzymes have still been reported from various sources and further studies are required for production scale-up of the proteins.
Aims: Here we report recent advances in our R&D facility on bioprocess development for novel antithrombotic enzymes purified from *Agkistrodon* and *Gloydius* species venom.
Methods: The purification process of disintegrin-like protein (DLP), phospholipase A_2 (PLA_2), protein C activator (PCA), fibronolytic enzyme (FE) and plasminogen activator (PA) involve centrifugation, filtration and a series of HPLC steps, such as ion exchange, reverse phase and size exclusion. The purity and activity of the proteins were determined using SDS-PAGE and Electron Spray Ionisation Mass Spectrometry, and enzyme electrophoresis, platelet aggregation assay and coagulation assay, respectively.
Results: The multi-step purification process efficiently removes process and product related impurities and results in high-purity as single bands in protein gel, with an average yield of $\sim$50%. The MS/MS analyses followed by Mascot search engine revealed identification and uniqueness of each preparation. Specific activities of ADP- or collagen-induced aggregation inhibition of rabbit platelets of the purified DLP, PLA_2, PCA, FE and PA fell in a range of between 210 U/mg and 570 U/mg. Final purified proteins were characterized to estimate molecular weight, p*I* and pH optimum.
Conclusions: Herein we document an efficient and robust production system for the antithrombotic proteins from *Agkistrodon* and *Gloydius* venom. The catalytically active proteins were conveniently purified in large quantities by using combination of liquid chromatography matrices. This study confirms that pilot scale laboratory grade antiplatelet agents can be produced in a cost-effective manner for further trials.

PP19

Real time dose adjustment utilizing point of care platelet reactivity testing in a double blind study of prasugrel in children with sickle cell anemia (SCA)

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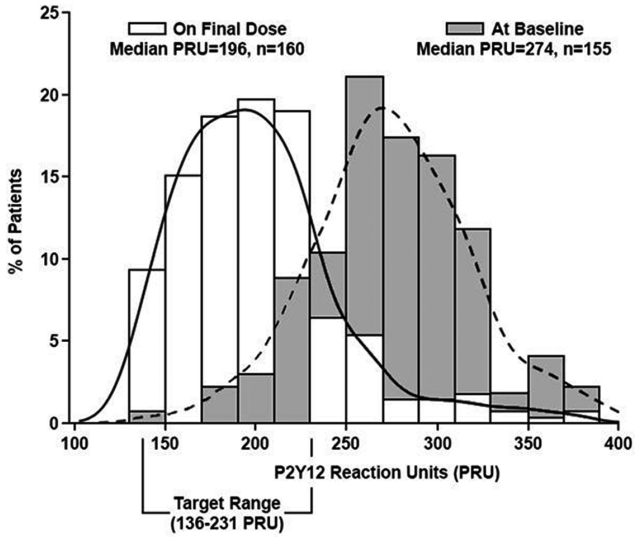
Background: ADP, released from sickled erythrocytes, has been implicated in platelet activation/aggregation which may contribute to ischemic pain in SCA. P2Y12 ADP receptor antagonists such as prasugrel may thus provide benefit. Previous studies suggested that a PRU (P2Y12 reaction unit) range of 231-136 would provide appropriate platelet inhibition, but individual dose adjustment would be required.
Aims: Real-time individual dose titration based on platelet testing as determined by PRU using Verify*Now*® P2Y12 (VN).
Methods: DOVE was a Phase 3 double-blind, placebo-controlled study of prasugrel in children with SCA. After baseline PRU determination by VN, patients started 0.08 mg/kg/day prasugrel. If after 14 days PRU values were outside of the 231-136 range doses were adjusted for an additional 14 days, and if necessary, a third dose adjustment was made. Maximum and minimum doses allowed were 0.12 and 0.04 mg/kg/day. To maintain study blinding, PRU data were encrypted and an interactive voice response system (IVRS) was used to determine the actual PRU value and instruct the investigator to maintain/adjust the prasugrel/placebo (mock titration) dose.
Results: Of 170 patients receiving prasugrel, 160 patients were titrated to a final dose (see Figure). Baseline PRU values ranged between 149 and 388. Dose lowering was required in 13% of patients and dose escalation in 23% of patients. After dose titration, 84% of patients had PRU values within range. PRU remained above 231 in 18 of the 34 patients receiving the maximum dose; these patients exhibited significantly higher PRU values at baseline than those who were titrated into the target range. Baseline PRU values correlated with the ultimate dose needed to achieve target PRU range ($r=0.4$, $P< 0.001$).
Conclusions: Although efficacy endpoints were not met, DOVE demonstrated that individual dose titration of antiplatelet therapy can be achieved in a double-blind study through use of encrypted point of care platelet testing and IVRS technology.



Figure

PP20

Measurement of platelet serotonin secretion by HPLC-electrochemical detection (ED) in a clinical hemostasis laboratory: sensitive, specific and accurate assay for diagnosing platelet secretion defects and heparin-induced thrombocytopenia

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Background: Diagnoses of mild platelet secretion defects (PSD) and heparin-induced thrombocytopenia (HIT) require accurate, reproducible, sensitive and specific assays. Here we assess HPLC with electrochemical detection (ED) to measure secreted serotonin (5HT), a specific and abundant constituent of δ-granules.

Aims: To assess the measurement of 5HT by HPLC-ED for diagnosing PSD and HIT.
Methods: 1. PSD: Light transmission aggregation is stopped adding cold EDTA/saline to the tube. Platelets were pelleted and frozen with distilled water and internal standard. After sonication and protein precipitation, 5HT was measured in dedicated instruments. 5HT measured in platelet pellets without agonists was considered 100%. Results were compared with the ^{3}H-5HT secretion assay.
2. HIT: washed normal platelets (Tyrode-PGE1) were resuspended in Tyrode, 2mM Ca^{++}, without PGE1. Controls (+ and -, provided by Dr. R.H Aster), and patients sera were inactivated (56°Cx30') and added to platelets with 0.1 and 100 IU of heparin or Tyrode buffer. After gentle stirring (1h, RT°) the reaction was stopped with PBS-EDTA and 5HT was measured in platelet pellets.
Results:

Table Results of PSD diagnosis.

Agonist	Reference range *N*= 39	Patients, normal PS. *N*= 181	Patients, inherited PSD *N*= 12	Patients, ASA-like defect *N*= 7
1 mM arachidonate	44±12	40±11	33±11	8±5
10 μM epinephrine	55±11	52±10	25±24	0.6±1.5
4 μM ADP	43±12	40±11	13±15	3±5
8 μM ADP	44±9	41±10	21±16	4±5
1 μg/mL collagen	39±10	36±11	9±7	2±2
2 μg/mL collagen	43±10	40±11	14±8	5±5

Detection sensitivity was 1ng/mL ($1x10^{7}$ platelets) with CV< 2%. HPLC-ED and ^{3}H-5HT were highly correlated (r=0.95, CI=0.94-0.96). Both assays had high concordances (0.91-0.97) and agreements (0.86-0.97) for all the agonists (*P*< 0.0001), which was absolut for epinephrine. Of note, PS with epinephrine was consistently higher than with other agonists (*P*< 0.0001). Bland-Altman plots showed no systematic bias between assays.

Table Results of 5HT secretion for HIT diagnosis.

	Controls, Negative	Controls, Positive	Patients, Negative	Patients, Positive
Median (range)	0% (0–17)	94% (78–94)	3.5% (0–16)	88% (86–92)
N° =	11	11	8	3

In HIT diagnosis, secreted 5HT measured by HPLC-ED strongly discriminates between negative and positive samples.
Conclusions: Routine HPLC-ED measurement of 5HT secretion to diagnose PSD, (assaying in parallel total 5HT content and platelet aggregation), is highly accurate, sensitive and specific. Despite our low number of cases, HPLC-ED appears to be also a robust assay for HIT diagnosis. (FONDECYT 1130853).

PP21

Assessment of platelet function on the routine coagulation analyzer sysmex CS-2000i

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Background: Light transmission aggregometry (LTA) is considered as the gold standard for testing platelet function in the setting of both platelet disorders suspicion and response to antiplatelet therapy evaluation. LTA requires however specialized equipment, substantial blood sample volumes, is technically challenging and time-consuming.
Aims: To evaluate an automated platelet aggregation method performed on a routine coagulation analyzer Sysmex CS-2000i.
Methods: 46 patients presenting a bleeding syndrome and 62 patients with acute coronary syndrome receiving dual antiplatelet therapy were studied in total. Platelet aggregations were performed on CS-2000i (Sysmex Corporation, Japan) equipped with a dedicated software and on APACT-4004 (Elitech, France) as the reference instrument. Aggregation was measured by monitoring the changes in light absorbance occurring in response to ADP 2.5, 5 and 10μM, collagen 3.3 μg/mL, epinephrin 10μM, ristocetin 1.25 mg/mL and arachidonic acid 0.5 mg/mL in platelet rich plasma (PRP). PRP were tested simultaneously on both CS-2000i and APACT-4004 devices. Platelet stirred speed were 800 rpm for both instruments.
Results: Significant correlations were observed between CS-2000i and LTA after all stimulations (*P*< 0.001). Patients presenting a bleeding syndrome had similar aggregation profiles with both methods. A single patient presented a severe platelet disorder (Glanzmann Thrombasthenia) and its PRP showed defective aggregation in response to all agonists except ristocetin with both instruments. Finally, the inter-agreement rates for CS-2000i and APACT-4004 to detect low responders to thienopyridines or aspirine were strong (weighted kappa> 0.61).
Conclusions: Platelet aggregation on the routine coagulation analyzer CS-2000i is an easily accessible, handy, reliable, standardized, and rapid tool to assess platelet function which allows to skirt most of the LTA limitations.

PP22

Platelet calcium flux measurement by flow cytometry in diluted whole blood

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Background: Hereditary platelet function disorders (PFD) have a broad aetiology with defects in adhesion, aggregation & secretion. Diagnosis of PFD involves time-consuming specialist techniques. As testing can be inconclusive, new laboratory methods are required to complement existing ones.
Aims: This study assesses the measurement of platelet calcium (Ca) flux by flow cytometry using a non-wash reagent as a potential technique for PFD diagnosis.
Methods: Citrated normal blood was diluted 1:10 in Ca-free Tyrode's buffer (CFTB), added to an equal volume of Fluo-4 & incubated in the dark (1hr). After transfer to 5mL of CFTB & mixing for 15min it was then diluted to record $0.2X10^{6}$ total events min^{-1} in an Accuri C6 flow cytometer. Fluo-4 median fluorescence intensity (MFI; 488nm laser, 530±15nm filter) was recorded from CD41 positive events as a function of time over 5min ± 1mM $CaCl_2$ adding 0.1, 1, 5, or 10μM ADP (final concentration) at 1min. Data were expressed as the ratio of peak MFI to baseline MFI. The effect of probenecid (PBN; 2.5mM) was also investigated.
Results: Reproducible baseline & peak MFI were obtained in 3 experiments using a single donor. In CFTB MFI ratios were identical with 1, 5 & 10μM ADP suggesting maximal internal Ca mobilisation. With Ca present, MFI ratios were increased & showed dose-dependent responses indicative of Ca influx. Responses to 0.1μM ADP were identical ± Ca (Figure 1).

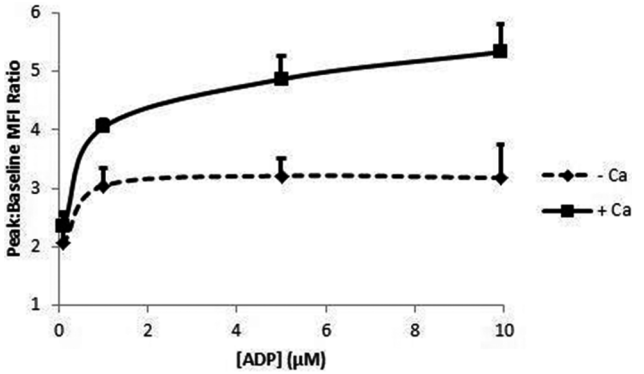

Figure 1

Labelling diluted whole blood with PBN present increased baseline & peak MFI values *vs*. untreated but significant loss of baseline MFI occurred over time (Figure 2).

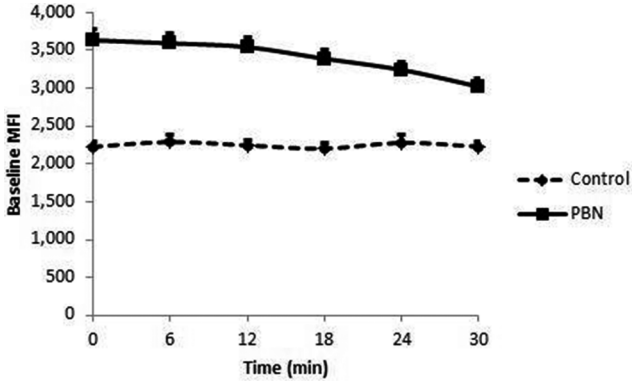

Figure 2

In response to 10μM ADP with Ca, the addition of PBN reduced the peak MFI ratio suggesting PBN inhibited Ca influx.

Conclusions: Diluted whole blood labelling with Fluo-4 offers a rapid & simple flow cytometric method to evaluate platelet Ca flux.

Internal Ca mobilisation alone or with Ca influx can be measured ± 1mM $CaCl_2$ respectively.

PBN increases Fluo-4 labelling at the cost of baseline MFI loss over time.

PBN inhibits Ca influx but not internal mobilisation.

PP23

Role of platelets in the spatial clot growth investigated with reaction-diffusion in vitro experimental mode

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Background: The known role of activated platelets is to accelerate blood coagulation by providing procoagulant membranes for the surface-dependent reactions. The integral effect of platelets on coagulation is a subject of debate due to existence of other membrane sources.

Aims: To investigate the role of platelets in the reaction-diffusion *in vitro* experimental model of spatial fibrin clot growth.

Methods: Coagulation was activated by immobilized tissue factor (TF) in recalcified platelet-rich plasma (PRP) or platelet-free plasma (PFP). Measurements of thrombin generation and spatial clot growth were performed using a pre-production model of Thrombodynamics-4D Analyzer System provided by HemaCore Labs where a videomicroscopic system registered the UV-LED excited fluorescence of thrombin-activated fluorogenic substrate and the light scattering signal from the growing fibrin clot. Statistical significance was estimated by pair-sample T-test.

Results: When coagulation was initiated by high TF density (80 pmole/m^2), platelets at 2×10^5/μL only moderately increased clot growth rate (V) from 30±1.7 to 35±2 μm/min (n=5, p=0.02). In contrast, the thrombin impulse was formed and propagated in a self-sustaining manner only in PRP but transformed to a diffuse form when platelet concentration decreased to 0.2×10^5/μL. Platelet inactivation with prostaglandin E1 completely abolished the thrombin impulse. In heparin-supplemented plasma (0.09 IU/mL), platelets increased V at high TF density from 13.1±1.5 in PFP to 31.8±1.4 μm/min in PRP (n=4, $P< 0.001$) returning it to the normal rate; notably, the thrombin impulse was also normalized. When the contribution of extrinsic tenase was reduced by decreasing the TF density to 8 pmole/m^2, platelets dramatically increased V from 6±2 μm/min in PFP to 43±10 μm/min in PRP (n=4, p=0.003).

Conclusions: Platelets stabilized the coagulation system making the spatial clotting process less sensitive to inhibitory (heparin addition) or low activation (low TF density) conditions.

PP24

Psoralen and ultraviolet A light treatment inhibits activation of PI3K dependent effector kinases by disturbing cell membrane packing

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Background: Psoralen and ultraviolet A (PUVA) light treatment is used for pathogen inactivation of platelet concentrates for transfusion. Systemic PUVA (extracorporeal photopheresis) is under evaluation for Graft- vs.-host-Disease (GvhD) after decades of clinical success in cutaneous T cell lymphoma. Despite this, PUVA mode of action remains poorly understood.

Aims: To demonstrate how PUVA impacts cell signal transduction.

Methods: Impact of PUVA was investigated by western blotting of (phosphorylated) signal transduction proteins, by mass spectrometry of phospholipids (PL) and by flow cytometry of (activated) platelet receptors. Lipid packing was measured with fluorescently labeled ALPS peptide.

T lymphocytes were activated with CD3/CD28 beads.

Results: Activation of integrin $\alpha_{IIb}\beta_3$ was decreased in PUVA treated platelets following dose escalated PAR1 and GPVI, but not PAR4 activation. Pleckstrin phosphorylation, calcium entry kinetics and Rap1b activation were normal. Membrane sublocalization and subsequent phosphorylation of Akt and Btk was significantly reduced in activated PUVA platelets, suggesting defective PI3K. However,

phosphorylation of inositides by PI3K was normal in all conditions. Mass analytical PL characterization uncovered covalent complex formation between psoralen and PL, exclusively on acyl chains with double bonds. Because acyl (un)saturations determine membrane packing, liposomes with different packing properties were prepared. PUVA treatment of poorly packed liposomes increased PL packing to levels typical of controls with saturated acyl chains. Decreased Akt phosphorylation was also found in healthy T lymphocytes treated with PUVA or in T cells from patients enrolled in a trial testing PUVA for GvhD treatment.

Conclusions: PUVA increases membrane packing by complex formation with PL acyl chains, likely causing the indirect but specific inhibition of PI3K dependent effector kinases.

PP25

Why platelets have two PARs?

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Background: The serine protease thrombin is in the center of both plasma and cellular hemostasis. Human platelets possess two main receptors for thrombin, protease-activated receptors (PARs) PAR1 and PAR4. Both are capable of invoking all possible platelet activation responses and they have identical main intracellular signaling pathways. Although there is experimental evidence that they have different cleavage/inactivation kinetics (and some secondary variations in signaling), the reason for such redundancy is not clear: why there must be two discriminate receptors?

Aims: Discrimination of the roles of PAR1 and PAR4 in platelet activation by thrombin.

Methods: We developed a multicompartmental stochastic computational systems biology model of dual-receptor thrombin signaling in platelets. Experiments employing continuous flow cytometry of washed, Fura Red-loaded and annexin-V labeled platelets were used to validate model and test its predictions. Investigations were performed in accordance with the Declaration of Helsinki, and written informed consent was obtained from all donors.

Results: The model described experimental data well; both receptors induced calcium spiking, with rapid and short-lived response from PAR1, while signaling by PAR4 developed slowly and propagated in time. Response of the dual-receptor system was both rapid and prolonged in time. PAR1 did not cause intracellular store depletion, while PAR4 did, which was followed by the activation of store-operated calcium entry. Different ratios of PAR1 and PAR4 led to different dynamics and dose-dependence of procoagulant platelet formation, which could explain the observed variability between donors.

Conclusions: The dual-receptor combination is critical to produce a response combining three critical features: sensitivity to thrombin concentration, rapid onset and steady propagation; specific features of the protease-activated receptors do not allow combination of all three in a single receptor.

PP26

Role of formyl peptide receptors in the regulation of thrombosis and haemostasis

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Background: Platelets possess formyl peptide receptors (FPRs), which were originally found on leukocytes where they play indispensable roles in the regulation of host defense and inflammatory responses. The expression of these receptors has been previously reported in platelets and their stimulation induces chemotaxis and calcium release in platelets.

Aims: In this study, we aim to determine the role of FPRs in the regulation of thrombosis and haemostasis using a range of *in vitro* and *in vivo* platelet functional assays. Furthermore, the regulatory mechanisms that control the functions of FPRs in platelets will also be established.

Methods: The ability of platelet activation induction by formyl peptides will be analysed using a range of platelet functional assays such as aggregation, granule secretion, calcium mobilisation, *in vitro* thrombus formation and clot retraction. Moreover, the anti-thrombotic properties of formyl peptides will be further scrutinised using similar approaches.

Results: The expression of FPR1 on human platelets has been confirmed by transcriptomics analysis and immunoblots. In addition, the flow cytometry analysis indicates that the level of FPR1 is increased upon activation of platelets. This suggests the presence of FPR1 in platelet granules. Similar observations were made in the previous study where the role of FPR1 in chemotaxis of platelets was demonstrated. FPR1 agonist, fMLF, induced platelet activation in a dose-dependent manner and its effects can be inhibited using FPR1 antagonists such as Boc-MLF. Moreover, CsH, an inverse agonist for FPR1, was used to assess its effects on CRP-XL induced platelet activation. Different concentrations of CsH reduced CRP-XL induced platelet activation in a dose-dependent manner.

Conclusions: Our preliminary data support our hypothesis that FPRs play a significant role in the modulation of platelet function.

PP27

Functional and molecular characterization of an inherited abnormal platelet function related to a new CalDAG-GEFI protein variant in an argentinean family

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Background: Signal transduction dysfunction is a cause of platelet-related bleeding. Genetic variants affecting the function of CalDAG-GEFI, essential for integrin activation, have only recently been identified and are ultra-rare.

Aims: Characterization of a platelet function defect in a pedigree with a bleeding diathesis.

Methods: The affected pedigree from Argentina consisted of two male siblings P1, P2 and their mother P3. Platelet aggregation was performed in a lumi-aggregometer. The agonist panel included:ADP,epinephrine(Epi),collagen(Col),TRAP,A23187,PMA,arachidonicacid (AA),ristocetin and thrombin(T). The monoclonal antibodies for flow cytometric (FACS) analysis were against CD42b, CD61 and CD41. The DNA samples were analysed using the ThromboGenomics high-throughput sequencing platform, which targets 113 genes.
Results: P1 and P2 have a lifelong bleeding syndrome with severe epistaxis, and hematomas requiring transfusions on several occasions. P3 had epistaxis during childhood, and menorrhagia. All had a normal platelet count. Platelet function testing demonstrated a much-reduced maximal aggregation for P1 and P2 and a normal aggregation for P3 in response to ADP, Epi, A23187 and at low doses of TRAP and Col; whereas aggregation with AA, ristocetin, PMA, high-dose Col and T was normal or slightly diminished. ATP release was absent with ADP, Epi or A23187 for P1 and P2 and marginally decreased with Col and AA. Membrane receptors aIIbβ3 and GPIb were present. Genetic analysis led to the identification of a novel homozygous c.914G>A transition in exon 9 of the *RASGRP2* gene for P1 and P2 and P3 was a carrier. This variant gives rise to p.Gly305Asp in CalDAG-GEFI.
Conclusions: We report a new variant p.Gly305Asp of CalDAG-GEFI identified in a pedigree with severe bleeding syndrome. The clinical phenotype resembled a Glanzmann's thrombasthenia variant but with residual platelet aggregation with strong platelet agonists and with PMA, a characteristic for this gene.

PP28

Thiol isomerase denitrosylase activity in platelet activation

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Background: A role for PDI, ERp5 and ERp57 in thrombus formation is now established and PDI antagonists are in clinical trials. Yet the mechanisms by which thiol isomerases contribute to thrombus formation are unknown.
Aims: To determine whether the denitrosylase activity of thiol isomerases contributes to its role in platelet activation and thrombus formation.
Methods: PDI active site variants were screened for variants with increased denitrosylase activity or increased reductase activity.
Results: Screening PDI active site variants identified one reductase-biased and two denitrosylase-biased variants. Using differential cysteine alkylation followed by mass spectroscopy to determine the redox potential of active site cysteines, showed that oxidase-biased variants had the highest redox potentials (E^o=-182 mV), while denitrosylase-biased variants had the lowest redox potentials (E^o=-252 mV). Incubation of platelets with a nitric oxide (NO) donor resulted in total inhibition of platelet activation. PDI, ERp5 and ERp57 reversed this NO-induced inhibition, while an enzymatically inert variant had no effect. Denitrosylase-biased variants were more effective than native PDI. The reductase-biased variant was less effective. We next evaluated the effect of PDI on nitrosylation of a2bb3. NO exposure resulted in a2bb3 nitrosylation. Subsequent exposure to PDI (or ERp5) resulted in denitrosylation of a2bb3. Activation-induced phosphorylation of the b3-subunit was diminished after NO exposure. PDI reversed NO-induced inhibition of phosphorylation, whereas the inert variant did not. Mice lacking GSNO reductase, an enzyme critical for metabolism of GSNO, demonstrated impaired platelet accumulation and fibrin formation in a model of *in vivo* thrombosis. PDI reversed this inhibition, while inactive PDI did not.
Conclusions: Redox potential is a critical determinant of PDI reductase vs denitrosylase activity and PDI denitrosylase activity functions in platelet activation and, potentially, in thrombus formation.

PP29

Value of whole blood impedance aggregometry in the diagnosis of inherited platelet function disorders

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Background: The gold standard test for the assessment of inherited platelet function disorders (PFD) is light transmission aggregometry in platelet-rich plasma, a long and delicate method requiring large blood samples.
Aims: Assess the value of whole blood impedance aggregometry (WBI) in PFD diagnosis, especially in case of associated thrombocytopenia.
Methods: Twenty-six patients with characterized PFD were studied including Glanzmann Thrombasthenia (GT, n=9), type 2B von Willebrand disease (VWD2B, n=7), platelet-type von Willebrand disease (PTVWD, n=3), Bernard-Soulier (BSS, n=3) and MYH9 syndromes (n=4). WBI was performed on hirudin- or citrate-anticoagulated blood on a Multiplate® analyzer (Roche). Aggregation was triggered using ristocetin (Risto0.77: 0.77 mg/mL, Risto0.32: 0.32 mg/mL), ADP (6.5 μM), collagen (3.2 μg/mL) and TRAP (32 μM). Results were expressed as area under the aggregation curve (AU). Normal ranges were based on 30 healthy volunteers' results.
Results: GT patients exhibited flat curves (< 97 AU) with all agonists except for collagen in 2 patients (340±150 *vs* 879±240 AU). Thrombocytopenic MYH9 patients (platelets: 52±14G/L) had sub-normal results with all agonists while BSS patients (platelets: 29±8G/L) had isolated flat curves with Risto0.77 (83±99 *vs* 1039±250 AU). In 2/3 PTVWD and all VWD2B patients, Risto0.32 showed significantly increased results (>300 *vs* 177±89 AU) despite low platelet count (20–71 G/L) in 4 patients.
Conclusions: In our study, WBI was able to 1) properly detect GT in case of flat traces with all agonists 2) diagnose BSS and VWD2B or PTVWD patients based on the ristocetin results, independently from the platelet count 3) exclude other PFD but MYH9 syndrome in case of normal WBI results, among 10 patients with platelet count < 70G/L. Our preliminary results thus suggest that WBI, which requires limited amounts of blood, is a sensitive, valuable, quick and easy-to-use method for the diagnosis of PFD even in case of associated thrombocytopenia.

PP30

Diagnostic value of a flow cytometry based platelet function test compared with light transmission aggregometry in patients with unknown bleeding disorders

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Background: Light transmission aggregometry (LTA) is the gold standard test for the diagnosis of platelet function disorders. Nevertheless, LTA is labor-intensive, is poorly reproducible and has moderate sensitivity for mild platelet function disorders. Therefore, we have developed a flow cytometry based platelet activation test (PACT) that measures fibrinogen binding to integrin αIIbβ3 and P-selectin expression as markers of platelet activation in response to agonist stimulation.
Aims: To compare the diagnostic value of the PACT with LTA.
Methods: Proof of principle of the PACT was tested in 10 patients with known platelet function disorders, including Glanzmann Thrombasthenia (GT), Storage Pool Disease (SPD) and Bernard Soulier Syndrome (BSS). For the validation of the PACT, patients (n=113) with a positive bleeding tendency (ISTH BAT score > 4) and suspicion of a primary hemostasis defect were included. Platelet reactivity upon agonist stimulation was determined with PACT and LTA, and compared with the platelet function of healthy controls (n=60). Furthermore, the reproducibility of the PACT and LTA was determined using healthy platelets and platelets with reduced reactivity.
Results: With the PACT, the platelet function disorders GT and BSS were readily identified based on an abnormal platelet response to agonist stimulation. Patients with SPD also showed abnormal platelet function in the PACT, but low platelet ADP content was required to confirm SPD diagnosis. With an area under the ROC-curve of 0.738 ± 0.038, the PACT showed increased discriminative ability between patients and healthy controls compared to the LTA (0.624 ± 0.043; p=0.048). Furthermore, PACT showed improved reproducibility (CV 5.8%) compared with LTA (CV 16.6%), especially in platelets with reduced reactivity.
Conclusions: The PACT was shown to be superior over LTA in discriminating between platelet function disorders and healthy controls, but also in test reproducibility, making PACT a promising new tool in platelet function diagnostics.

PP31

Antiplatelet aggregation activity of two schiff base ligands derived from 3-hydroxy-2-naphthaldehyde and their corresponding copper(II) complexes

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Background: Polyphosphates (PolyPs), released by platelets, promote a procoagulant activity: stabilize fibrine, activate factors V, XI and X. PolyPs released by microorganisms, with long chains, activate FXII and increase the bradykinin production and inflammation. The increased procoagulant activity through the above mechanisms may lead to thrombosis. There is an increasing interest in drugs that may interact with PolyPs, such as the Schiff base copper(II) complexes, for its possible antiplatelet-anticoagulant activity.
Aims: Synthesize and characterize the antiplatelet aggregation activity of two tridentate Schiff base ligands derived from 3-hydroxy 2-naphthaldehyde, 2-[N-{pyridin-2-yl-ethylimino}methyl)]-3-naphthol (**1**) and 2-[N-({2-morpholin-4-yl-ethylimino}methyl)]-3-naphthol (**2**), and, their corresponding copper(II) complex (**3**) and (**4**) respectively.
Methods: Blood was collected by venipuncture to blood bank donors of the National Institute of Cardiology. The samples were maintained at room temperature for no more than 30 min, and they were gently mixed immediately before the whole blood platelet aggregation was initiated by pre-incubating the samples for 3 min at 37°C in an aggregometer (AGGRO/LINK Chrono-log, Havertown, USA). The compounds (5-50–500 μM) were dissolved in ethanol and were added to the blood to a total volume of 5 μL. The results obtained with ethanol were considered as a control for 100% platelet aggregation and 0 % platelet inhibition.
Platelet aggregation was induced with 5 μM ADP,the response was recorded over the course of 5 min. The study was approved by the institutional ethical committee.
* $P < 0.05$ Dunnet test.
Results:

Table 1 Percentage of platelet inhibition.

Compound	Vehicle	5 μM	50 μM	500 μM	IC50 (μM)
2,3NetPy (1)	0 ± 2.7	-6.1 ± 7.7	27.0 ± 11.6	7.9 ± 14.9	111
2,3Netmorph (2)	0 ± 2.6	-1.9 ± 11.9	11.5 ± 9.1	17.7 ± 4.7	45
Cu-2,3NetPy (3)	0 ± 4.2	5.7 ± 7.0	0.5 ± 4.7	12.6 ± 8.2	325
Cu-2,3NetMorph (4)	0 ± 1.9	10.8 ± 0.5	40.1 ± 8.4 *	41.1 ± 4.8 *	8.2

Conclusions: Compound (4) inhibit platelet aggregation induced by ADP. The presence of Cu in the molecule favors a rigid structure enabling its direct interaction between the phosphate polymer through van der Walls forces with the oxygen of morpholin.

PP32

TPO agonist for the treatment of may hegglin anomaly deriving from MYH9 mutation

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Background: May-Hegglin anomaly (MHA) is a rare autosomal-dominant thrombocytopenia caused by mutation of MYH9, the gene for the heavy chain of myosin-IIA (NMMHC-IIA). MHA is characterized by thrombocytopenia, giant platelets, and aggregates of the mutant protein in leukocytes, which are often evident upon conventional stainings as basophilic "Döhle-like" inclusions. Deafness, cataract and glomerulopathy have also been described.
Aims: Platelet transfusion is currently the primary therapeutic option for reducing thrombocytopenia in cases of hemorrhagic syndrome, and eventually TPO agonists, which appear to be effective in MYH9-related disease (Pecci et al., 2010).
Methods: We report the case of a young girl of 15 who has presented himself for investigation of a hemorrhagic syndrome goes back to childhood marked by epistaxis and petechiae, which has worsened after the ménarchie by menorrhagia rebels to any therapy.
Results: The examination of the peripheral blood smear confirmed the thrombocytopenia, and showed giant platelets and body Dohles in neutrophils. May Hegglin disease was discussed then confirmed by mutational PCR analysis c.4261G finding the mutation> A in exon 30 of the gene MYH9.View non-response to platelet transfusions and aggravation of hemorrhagic syndrome, we decided to treat our patient who had a platelet count of 5 G/L by a TPO agonist (Romiplostim) at a rate of 5 μg / kg / week, after 2 weeks we got a very good clinical

and biological response with a platelet count that is maintained between 70G / 100G/L.
Conclusions: This simple observation has allowed us confirm the performance of TPO agonists in thrombopathy of May Hegglin, however, this treatment can not be continued long enough because of serious side effects have been described as pulmonary fibrosis.

PP33
In vitro antiplatelet activity of anthocyanin

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Background: Increased platelet activity plays a significant role in the development of arterial thrombosis and cardiovascular disease (CVD). There is strong evidence of aspirin(common cardio preventive antiplatelet therapy) resistance in people with diabetes. Natural antioxidants including anthocyanin (AC) have gained great interest due to their hypothesized anti-thrombotic potential.
Aims: The aim of our study was to examine the *in vitro* effect of pure AC compounds on the platelet activity and aggregation.
Methods: Fasting blood sample was collected from screened thirteen healthy volunteers after obtaining ethics clearance and signed informed consent. A full blood examination and a dose response curve were performed by incubating platelets with 5 concentrationsof AC (25–200 mg/L). Platelet activity was assessed byflow-cytometer by recording platelet surface markers expression of activation-independent (CD41a) and dependent (P-selectin, CD62p). Platelet aggregation studies were performed by stimulating platelets using three different agonists ADP, collagen and arachidonic acid (AA).
Results: Anthocyanin (50 mg/L) significantly inhibited AA-induced platelet aggregation. ADP and collagen stimulation also resulted in significantly decreased platelet aggregation but at higher concentrations. Expression of P-selectin was significantly suppressed by 50 mg/L AC as measured by the decreased platelet surface expression of CD62p by flowcytometer.
Conclusions: Our results demonstrate that AC may be instrumental in attenuating platelet aggregation by suppressing P-selectin expression and influencing Thromboxane-A2 pathway (AA stimulation) with similar mode of action as Aspirin. These results provide further evidence to the effect of AC and possible mechanism by which AC reduces platelet aggregation and activation. This *in vitro* study supports future human intervention trials to show that AC may act as a complement other anti-platelet agents in reducing risk of thrombosis particularly in aspirin resistant diabetes population.

PP34
Sticky platelet syndrome - questionable or independent risk factor for thrombosis?

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Background: The sticky platelet syndrome (SPS) is defined by its clinical and laboratory features. The clinical symptoms of SPS are characterized with thromboembolic events (TE) and as followed: a) young adults (< 40 years) without known risk factors, b) pregnant woman often affected; association with fetal loss syndrome, c) often atypical localization of thrombosis, d) both arterial (more often) and venous thrombosis presented, e) recurrent thrombosis during adequate anticoagulation therapy, f) often positive family history for TE. According to diagnostic criteria, SPS is a hereditary thrombophilic thrombocytopathy defined by an increased *in vitro* platelet aggregation after low concentrations of adenosindiphosphate and/or epinephrine.
Aims: The aim of the work is to summarize the research on SPS in the literature and to provide the authors' own clinical and diagnostic experience.
Methods: Authors introduce current research knowledge in SPS and their own data about SPS from National registry of thrombophilic states in Martin, Slovakia.
Results: 361 cases with SPS positivity were confirmed in cohort of 1704 patients with unexplained thrombotic event or fetal loss tested for SPS. Twenty percent of the patients with SPS were younger than 30 years (5% younger than 20 years). SPS was manifested in 47% of patients with arterial thrombosis, 39% had venous thrombosis and 14% fetal loss syndrome, respectively. It is of interest that there was more than 20% patients with SPS positivity in the group tested due to migraine.
Conclusions: Family studies of patients with SPS showed that some of their relatives fulfilled laboratory criteria for SPS but remained clinically asymptomatic. It seems, that acquired risk factors for thrombosis may be crucial for the clinical manifestation of SPS. The relation between the onset of TE and stressful situation in SPS, supports this idea. The study was supported by projects Vega 1/0168/16, APVV 0222-11 and BioMed Martin, ITMS 26220220187.

PP35
An atypical IgM antibody that induces integrin $\alpha_{IIb}\beta_3$ activation and glanzmann thrombasthenia-like phenotype associated with macrothrombocytopenia

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Background: Acquired Glanzmann thrombasthenia (GT) is a rare bleeding disorder generally caused by anti-$\alpha_{IIb}\beta_3$ auto-antibodies.
Aims: Here we report the case of a patient who has progressively developed a GT-like phenotype associated with macrothrombocytopenia.
Methods: Measured were:
i platelet aggregation to different agonists,
ii platelet glycoproteins expression and
iii presence of anti-$\alpha_{IIb}\beta_3$ antibodies.
Results: The patient is a 50-year-old man with chronic ITP. After unsuccessful splenectomy, romiplostim was initiated and rapidly increased his platelet count. However, in July 2013, he suffered from repeated episodes of gastrointestinal bleedings and further studies indicated a moderate platelet aggregation defect. In December 2013, platelet function testing showed much reduced aggregation using all agonists, but normal agglutination with ristocetin. Expression of $\alpha_{IIb}\beta_3$ was markedly reduced and there was nearly no activation of GPIbα or P-selectin upon platelet stimulation. An excessive presence of giant platelets seen in the patient suggested that this signal transduction defect has also an effect on platelet production.
The presence of an anti-$\alpha_{IIb}\beta_3$ antibody was identified in the patient serum, yet platelet surface-associated antibodies were negative. Surprisingly, an IgM class antibody was strongly detected in

permeabilized platelets, showing that this antibody was internalized and further stored into megakaryocytes. Platelet fixation of the immunoglobulin was dependent of $\alpha_{IIb}\beta_3$ as no fixation was observed with washed platelets from GT type I patients. Platelet mixing study also showed activation of the $\alpha_{IIb}\beta_3$ complex as evidenced by increased PAC-1 binding to controls' resting platelets.
Conclusions: In conclusion, we present the case of a patient with a severely perturbed platelet function associated with the presence of an IgM activating auto-antibody directed against $\alpha_{IIb}\beta_3$.

PP36

Membrane microvesicles generated in stored blood components

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Background: There is growing evidence on the clinical importance of MVs and their role in blood transfusion. Stored Blood bank components: red blood cells (RBC), apheresis platelet concentrates (A-Plts), fresh frozen plasma (FFP) and cryoprecipitate (Cryo), may contain an increased number of microvesicles (MVs).
Aims: To quantify MVs in stored blood bank components.
Methods: Blood was obtained from healthy donors in accordance with the ethical standards of the local research committee and with Helsinki declaration. Blood bank components (n=80) were obtained and stored under strict controlled conditions (National Institute of Cardiology, Regulation NOM-253-SSA1-2012). A time course of storage-induced MVs release was tested for each component (RBC, A-Plts, FFP and Cryo). MVs were first isolated by sequential centrifugation at 20000 g for 90 min at 4°C. The isolated MVs were then lysed by thermic shock and their protein content measured at A280 nm. MVs' concentration was further estimated considering that a 1 μg/mL protein = $8x10^5$ Mvs/μL to Freyssinet and Toti. Data are presented as median (minimum-maximum).
(Wilcoxon test, SPSS v21.0 software).
Results: There was statistical difference in RBC, A-Plts and Cryo. MVs concentration derived from cell membranes of blood components increases significantly with storage time. Results are summarized in the table below.
Conclusions: Procedures used for the isolation and quantitation of MVs ensure the correct evaluation of the total amount of MVs in the different blood bank components tested. RBC and A-Plts-derivated MVs increase in number in a time dependent manner during storage. In Cryos the number of Mvs increases only at Day 60, most probably due to the presence of precipitated platelets.
These results raise the question about the clinical consequences of this event when these blood components are transfused.

PP37

Atabrine binds to the platelet $5HT2_A$ receptor and is not taken up into platelet dense granules

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Background: The original mepacrine became unavailable and atabrine with "mepacrine" listed as a synonym was offered as an equivalent reagent for the mepacrine assay.
Aims: Our objective was to replace mepacrine with atabrine in the mepacrine assay but markedly different assay conditions were required for atabrine suggesting different modes of action for each reagent. We hypothesised that mepacrine and atabrine act via different serotonin receptors and we utilized serotonin receptor blockers, palonosetron a potent $5HT3_A$ antagonist and reserpine a $5HT2_A$ inhibitor to identify receptor targets.
Methods: We developed and optimized the atabrine assay. We examined atabrine uptake into red cells because mepacrine is taken up into red cells for the anti malarial destruction of P falciparum gametocytes. We carried out blocking studies using 10 μL palonosetron (250 μg in 5 mL) or 10 μL of 30 μM reserpine. Blockers were incubated with platelets for 20 min at 37°C prior to the assay that was modified to reduce the influence of red cells on blocking efficiency.
Results: Atabrine incubation time was reduced to 15 min because atabrine bound to the $5HT2_A$ receptor fell away from the platelet membrane. There was minimal uptake into red cells and atabrine required at least 10 fold dilution to demonstrate dose dependent binding increments. Assay blocking was calculated as percent of unblocked in the mepacrine assay 10 μL palonosetron 75±9% (N=10) and for 20 μL 61±8% indicating prozone effect. Granisetron blocking studies are in progress. Reserpine blocking was 80±6% in the atabrine assay and 20 ± 5% in the mepacrine assay (N=5).
Conclusions: Mepacrine and atabrine assays measure different platelet functions. The optimized atabrine assay should be useful as a diagnostic tool to detect impaired binding and cell signalling related to $5HT2_A$. Mepacrine is taken up into dense granules via the ion channel receptor $5HT3_A$.

PP38

Aspirin Response in Myeloproliferative Patients using Multiplate Aggregometry

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Background: Multiplate electrode aggregometry (MEA) is a validated method of assessing aspirin effect and other platelet functions. The prevalence of aspirin resistance is approximately 21% in general population, but there is little data on aspirin sensitivity in patients with Myeloproliferative disorders (MPNs). MPN patients are at risk of thrombotic and haemorrhagic complications, and aspirin is in routine use to reduce thrombotic risk. Resistance to aspirin could therefore increase risk of thrombotic complications, and also increase haemorrhagic risk in those with platelet dysfunction.
Aims: The aims of this study were: 1) to establish percentage of patients with MPNs who show aspirin resistance.

Table Results of blood components-derived MVs. (Abstract PP36)

RBC ($x10^8$MVs/μL)		A-Plts ($x10^8$MVs/μL)		FFP ($x10^8$MVs/μL)		Cryo ($x10^8$MVs/μL)	
Day 1 (*n*=20)	Day 42 (*n*=16)	Day 1 (*n*=20)	Day 5 (n=20)	Day 1 (n=20)	Day 60 (n=8)	Day 1 (n=20)	Day 60 (n=20)
0.974 (0.178 - 3.790) *P*<0.05	2.894 (0.576–9.70)	1.082 (0.396–4.064) *P*< 0.05	3.932 (1.716–18.453)	0.535 (0.114–1.534) P > 0.05	0.381 (0.16–1.016)	1.267 (0.106–3.764) P < 0.05	1.901 (0.744–7.214)

2) to assess for other platelet function defects in this group.
Methods: Adults with MPNs (Essential thrombocythaemia, ET, and Polycythaemia Rubra Vera, PRV) were enrolled during clinic attendance following consent. A total of 92 samples were collected into bottles anticoagulated with hirudin and analysed within an hour of sampling. The samples were analysed for: aspirin response, in addition to rsponses to: collagen, ADP and TRAP.
Results: Overall response rate to aspirin was 22.2%, similar to the general population. The mean platelet count in responders was 359.3 as compared to 478.6 in non-responders. All patients had a response to TRAP, which acted as a further control; About half had reduced response to COL but all had normal response to ADP.

Table 1

	Total	*JAK-2 +ve*	*JAK-2 -ve*	*CAL-R +ve*	*COL↓*	*ADP↓*	*TRAP↓*	*ASPI response*
PRV	32	29	3	N/A	15/29	0/29	0/32	23/31 (25.8%)
ET	50	30	20	6	25/50	0/50	0/50	40/50 (20%)
Controls	10	6	4		0/10	0/10	0/10	
Total	92							

Conclusions: Aspirin response rate is similar in MPN patients to general population; a significant number had reduced response to collagen. Platelet count appeared to affect likelihood of response to aspirin, with higher counts less likely to respond. MEA may have clinical utility in assessing platelet response to aspirin and platelet dysfunction in MPN patients.

PP39

Influence of exercise intensity on platelet aggregation in patients with heart disease patients

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Background: Although exercise training is essential to treat ischemic heart disease, occurrence of coronary arterial thrombosis during exercise training must be avoided. Measurement of platelet aggregates with a particle counting method using light scattering is a useful method to evaluate the activity of small particles. However, it is not fully studied yet about the effect of exercise intensity on platelet aggregation.
Aims: Hereby, we planed to evaluate the effect of exercise intensity on platelet aggregation in patients with heart disease.
Methods: Twenty heart disease patients (66+-9 yrs old) performed cardiopulmonary exercise testing (CPX). At rest, at anaerobic threshold (AT) and at the peak exercise, blood sampling was collected and platelet aggregation was evaluated using platelet-rich plasma. A particle counting method using light scattering was used, and multiplication level of the small particle (50–70 nm) of platelet was measured.
Results: Patients' average AT and peak VO2 were 1.1+-4.1 and 17.9+-4.2 mL/min/kg. Total amount of small particle at rest, AT and peak exercise were 18.3+-11.7, 16.0+-14.3 and 28.7+-19.2* × 1000, respectively (*:$P< 0.01$, one-way ANOVA and Fisher's analysis).
Conclusions: It was revealed that small particle of platelet aggregation was not enhanced at the intensity of AT. Exercise training at the intensity of AT seemed to be safe in the point of platelet aggregation.

PP40

Platelet function diorders diagnosis using flow cytometry in algeria: about 19 families

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Background: Thrombopathies are rare bleeding disorders mostly diagnosed using platelet function analysis. However, the diagnosis may be difficult in some cases notably in poatients with profound thrombocytopenia that enabled platelet function testing, as well as the identification of heterozygotes in whom platelet function is normal. In these case only the flow cytometry allows the analysis of platelet antigens expression. We report the study of 19 families, with congenital platelet function disorders, by flow cytometry.
Aims: Diagnosis of congenital thrombopathies using flow cytometry.
Methods: Platelet count, platelet function testing by agregometry using five agonists (ADP, Collagen, Epinephrin, Arachidonic acid and ristocetin) and flow cytometry analysis using the detection and the quantification of CD41, CD61, CD42a, CD42b, CD63 and CD62P on quiescent and stimulated platelets.
Results: Bernard Soulier Syndrome was confirmed in 09 families, although thrombocytopenia encountered in the proband, the flow cytometry analysis showed a total absence of CD42a and CD42b. Glanzman thrombasthenia was found in 08 families, the flow cytometry detected heterozygote siblings presented with decreased GpIIbIIIa sites number despite of a normal platelet function testing. Moreover, 02 families showed a qualitative defect in the platelet GpIIbIIIa with no increase of the expression of this glycoprotein on stimulated platelets. Nevertheless, 02 patients had no expression of the CD63 on stimulated platelets consistent with a delta storage pool desease.
Conclusions: Flow cytometry is a decisive tool in the diagnosis algorithm of platelet membrane glycoproteins defects.

PP41

Predictors of the secondary aspirin resistance in patients with coronary artery disease over 60 (according to the 10-year follow-up)

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Background: Work carried out in the framework of the prevention of thrombotic complications in the elderly.
Aims: The study of the effectiveness of antiplatelet therapy is very important for the prevention of thrombotic events in patients with cardiovascular disorders.
Methods: It analized data agregatometry and medical history in 1789 patients 60–89 years with coronary heart disease. All of them long and regularly took ASA in a dose of 75–100 mg / day. The observation period 2000–2010 year. All patients where determined platelet aggregation in the two-channel laser analyzer 230LA («Biola", Russia) turbidimetric method. We assessed the level of spontaneous and induced platelet aggregation (ADP, epynefrine).
Results: As a result of the retrospective analysis, in patients aged 60–89 years with coronary heart disease, initially sensitive to acetylsalicylic acid, resistance to the drug after 1 year of observation developed in 12% of patients, after 2 years - 21%, in 5 years - 39%.
Modified risk factors of secondary resistance to acetylsalicylic acid: the level of cholesterol in blood plasma over 7.0 mmol / l - OR = 4.51 [95% Cl 3.60–5.64], diabetes mellitus - OR = 1.92 [95% Cl 1.51–2.43], glucose levels above 8.5 mmol / n- OR = 3.75 [95% Cl 2.86–4.92], left ventricular ejection fraction < 50% - OR = 1.61 [95% Cl 1.27–2.40], smoking more than 10 cigarettes a day OR = 3.23 [95% Cl 2.60–4.02] microcirculation disturbance 1.1 ml • min-1 / 100 g - OR = 1.29 [95% Cl 1,03-1,62], the level of endothelium-dependent vasodilation < 4.0 ml • min-1/100 g - OR = 2.55 [95% Cl 2,02 -3,21].
Conclusions: In 39% patients, who long administration of ASA antithrombotic efficacy of treatment is reduced. It requires timely laboratory testing and correction therapy.

Predictive/Diagnostic Variables

PRE01

Transient impact of treatment exposures and one-year incidence of thrombosis in multiple myeloma: a case-time-control analysis

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Background: Multiple myeloma (MM) has an inherent high risk of thrombosis of nearly exacerbated by specific treatment modalities.
Aims: This study sought to assess the acute, transient impact of treatment-related exposures on the risk of thrombosis in MM.
Methods: A case-time-control (CTC) analysis was conducted within a larger cohort of patients with MM. Individuals were identified by the first inpatient primary diagnosis of MM (ICD-9-CM 203.xx) from administrative claims during 2008–2013. Individuals included were over the age of 18 with continuous enrolment for 6 months preceding the index date. Subjects were followed until loss to follow-up, death, or a thrombotic event occurred (deep vein thrombosis, pulmonary embolism, arterial thrombosis, portal vein thrombosis). Cases included 502 subjects with at least 90 days of look-back preceding the thrombosis event. Cases were matched 1:4 with controls based by the year of MM diagnosis and controls were assigned the same event date as the case. Exposures were assessed in hazard (1–30 days) and comparison (61–90 days) periods preceding the event for cases and controls. Conditional logistic regression was used to compute adjusted odds ratios (aOR) for the transient effect of exposures on thrombosis. Exposures of interest included thalidomide/lenalidomide (IMIDs), protease inhibitors (PIs), steroids, cytotoxic agents, stem cell transplant, hospitalizations, and anticoagulation.
Results: The cohort included 13,700 individuals with 1,050 thrombotic events - a rate of 107.2 (100.9–113.9) per 1,000 person-years. The CTC analysis showed transient risk associated with IMIDs used alone (aOR=1.5 [1.1–2.1]) or with PIs (aOR=1.6 [1.0–2.6]). Stem cell transplant had the highest transient impact on thrombosis (aOR=3.7 [3.3–4.2]). PIs alone had a lower impact on thrombosis (aOR=0.8 [0.5–1.4]).
Conclusions: CTC results identify exposures with increased transient risk where surveillance and prophylaxis may be most useful.

PRE05

Acute isolated superficial-vein thrombosis is associated with substantial comorbidity and history of venous thromboembolic complications

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Background: The reported incidence of superficial-vein thrombosis (SVT) of the lower extremities is estimated to be greater than that of deep-vein thrombosis, and patients with SVT may be at substantial risk of symptomatic thromboembolic complications without appropriate care. CALISTO, a randomized, controlled trial in 3002 patients with isolated SVT, demonstrated significant clinical benefit with anticoagulant therapy (fondaparinux) vs. placebo.
Aims: We aim here to compare demographic characteristics, medical history, and ultrasonographic description of the thrombus in patients with isolated (without concomitant deep vein thrombosis) SVT in current ambulatory practice with those of the CALISTO trial population.
Methods: PERSEUS was a nationwide (France), observational cohort study to describe the current medical and surgical therapies utilized for patients with acute, isolated SVT of the lower extremity and to assess the incidence of thrombotic and bleeding events at 3 months in the real-world, ambulatory care setting.
Results: 978 patients were included. Demographic parameters, medical history and description of the index event are shown (see Table) from this study and from the CALISTO trial. Ninety percent of patients included in PERSEUS were prescribed some form of anticoagulation, predominantly fondaparinux.
Conclusions: In a real-world setting, patients with SVT were older, had greater comorbidity, and more often had a past history of venous thromboembolic events than in the CALISTO trial. These findings support the inclusion of SVT, along with deep vein thrombosis and pulmonary embolism, as part of the conceptual paradigm of venous thromboembolic disease.

Table Baseline characteristics in PERSEUS and CALISTO*.

Characteristics	CALISTO (N=3002)	PERSEUS (N=978)
Age (mean age in years)	57.0	62.9
Sex (% female)	63.9	64.2
BMI (mean in kg/m2)	29.1	27.3
Past history of SVT (%) (>3 months before inclusion)	11.9	34.2
Past history of DVT or PE (%) (>3 months before inclusion)	7.0	17.8
Active cancer (%)	0 (exclusion criterion)	3.2
History of cancer (%)	2.0	6.9
Varicose veins (%)	88.6	92.8
Above-knee SVT (%)	46.8	38.4

*Decousus H et al. N Engl J Med 2010;363:1222–32.

PRE07

Developpment of a new risk assessment model for VTE specific for ambulatory patients with lung adenocarcinoma on chemotherapy. The ROADMAP study

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Background: In ambulatory patients with advanced or metastatic stage of lung adenocarcinoma (LA) the risk of VTE increases during chemotherapy. but individual risk factors cannot identify patients at risk. The available RAM are not applicable in patients already on chemotherapy.
Aims: The prospective longitudinal non interventional study ROADMAP was designed to elaborate a RAM for VTE specific for ambulatory patients with LA on chemotherapy.
Methods: Patients with LA on chemotherapy were included and followed up at 3, 6 and 12 months. Documented symptomatic VTE was the end-point of the study. Blood samples were collected at inclusion

and assessed for thrombin generation (TG) and procoagulant phospholipids (PPL-ct). Assays and reagents were from Diagnostic Stago (France). Multivariate analysis was performed and the RAM was developed using the logistic regression. Sensitivity, specificity and the predictive value of the RAM were calculated. The ROC Curve was plotted.

Results: The study included 150 patients (mean age 65 years, 73% male). The LA diagnosis was done within 6 months before inclusion in 70% of them and 90% had advanced or metastatic disease at inclusion. In 85% of patients the ECOG performance status was < 3. In one year follow up 12 symptomatic VTE episodes occured (8%), 75% of which occurred within the 3 months from inclusion. The RAM includes the following variables: Recent hospitalisation, Time since diagnosis of the cancer, Mean Rate Index of TG and PPL-ct. The ROC analysis gave a AUC value of 0.84. The sensitivity of the RAM was 89% and the specificiy was 70%. The postive predictive value is 16% and the negative predictive value is 98%.

Conclusions: The new RAM for VTE is specific for ambulatory patients with LA on chemotherapy and can relibly predict VTE using simple clinical variables and biomarkers of hypercoagulability. This RAM can be used by physitians for the identification of ambulatory lung cancer patients eligible for thromboprophylaxis.

PRE08

Prevalence of hemostatic defects in dengue hemmorhagic fever in a tertiary care hospital in karachi

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Background: Dengue is a mosquito borne viral infection in the tropical and sub-tropical regions of the world. Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF) are two clinical manifestations presenting severe thrombocytopenia with mild cutaneous hemorrhages.The DHF is characterized by plasma leakage and shock due to increase in vascular permeability leading to defects in the first phase of haemostasis. Exact cause is unkown.

Aims: Observe changes in blood parameters in DHFpatients.

Methods: Cross sectional study was conducted over a period of 2 years from 2013 to 2014 at National Institute of Blood Diseases & Bone Marrow Transplantation, Karachi.306 dengue suspected patients of either sex were included. A clinical examination with detailed history was conducted. Dengue was confirmed by serology. The complete blood picture (CBC), prothrombin time,activated partial thromboplastin time, fibrinogen,D-Dimer, liver function test were performed.Screening for malaria, typhoid, HCV, HBV infections also done.

Results: Out of 306 cases 96 were confirmed as DHF. 95.8% patients presented with myalgia and 88 had headaches. No organomegaly. Serologic antibodies found in all patients.Average platelet count $47.2x10^3$ /ul and $TLC5.3x10^3$/ul .83 patients had prolonged PT while 92 patients had prolonged APTT value.Total bilirubin raised in 7 patients, ALK phosphatase in 91 patients and SGPT in 87 patients. Highly raised D-Dimer values recorded in 96% cases while only 12% patients had higher fibrinogen levels. No co-morbidity was observed.

Conclusions: Marked haematological abnormalities were observed in all the patients of DF regardless of age, sex and clinical presentation. Haemostatic abnormalities were observed in patients suffering from DHF.

PRE09

Validation of the ISTH-SSC bleeding assessment tool for the diagnosis of inherited bleeding disorders: experience from a tertiary care center in South India

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Background: A detailed bleeding history is essential to distinguish symptoms that are abnormal from those that are normal.A number of

Table 1 Type of presentation and average BAT score. (Abstract PRE09)

Diagnosis(n)	Epistaxis/ Cutaneous bleeding n(%)	Bleeding from minor wounds n(%)	GI bleeding n(%)	Oral cavity bleeding / Bleeding after Tooth extraction n(%)	Bleeding after Surgery or Major Trauma n(%)	Menorrhagia n(%)	Muscle hematomas (spontaneous) / sis n(%)	Other ings)	Average BAT score
Factor VIII/IX deficiency(27)	-/7(25.9)	8(29.6)	-	7(25.9)/-	8(29.6)	-	16(59.2)/19(70.3)	8(29.6)	7.6
Factor /2(100)	2(100)	2(100)	-/-	-	-	-/-	2(100)	6.5	
Factor /2(100)	2(100)	2(100)	-/-	-	1(50)	-/-	-	9.5	
Factor /-	-	-	-/1(100)	-	1(100)	-/-	-	8	
Factor /1(50)	-	1(50)	-/-	-	-	-/-	1(50)	9	
VWD(9)	6(66.6)/ 4(44.4)	-	-	6(66.6)/-	-	6(66.6)	2(22.2)/-	-	6.6
GT(4)	1(25)/ 4(44.4)	-	-	2(50)/ 1(25)	-	1(50)	-/-	-	9
BSS(1)	1(100)/ 1(100)	-	-	-/1(100)	-	-	-/-	-	6
Normal screening coagulogram(33)	11(33.3)/ 2(6.0)	-	4(12.1)	9(27.2)/-	3(9.0)	11(33.3)	-/-	-	3.8

bleeding assessment tools (BATs) have been developed to standardize the bleeding history to improve diagnostic accuracy thus avoiding unwarranted laboratory testing.

Aims: The aim of the study was to test the diagnostic utility of the ISTH-BAT for inherited bleeding disorders.

Methods: This was a cross-sectional study over 10 months (March 2015 and Jan 2016) wherein 89 consecutive patients suspected to have inherited bleeding disorders referred to our coagulation lab (Department of Pathology, JIPMER,Pondicherry,India) and 30 healthy volunteerswere enrolled who were administered ISTH-BAT. BAT score and screening tests were used to distinguish between primary and secondary hemostatic defects. Confirmatory tests were done in all secondary hemostatic defects and some tests for primary hemostatic defects are in progress.

Results: The median age of the patients was 12 years (2 months-69 years) and included 49 males and 40 females. Eight cases turned out to be acquired coagulopathies which were excluded. Secondary hemostatic defects were seen in 34 patients which includedHaemophilia A(25),Haemophilia B(2), Factor I deficiency(2), Prothrombin deficiency(2),Factor X deficiency(1) and Factor XIII deficiency(1). Primary hemostatic defects were suspected in 47 patients comprising platelet function disorders (1 BSS and4 GT), vWD(9)and those with normal screening coagulogram(33). The patients with normal screening coagulogram are being worked up to exclude mild VWD. The average BAT score of healthy volunteers was accounted to be 0.6. The clinical presentation and average groups is summarized in Table 1 on the previous page.

Conclusions: Obtaining a detailed history is crucial to evaluating a potential bleeding disorder. Because clinical symptoms can be subtle in patients with mild bleeding disorder, a standardized and quantitative approach, using questionnaires to calculate bleeding scores should be evaluated.

PRE10

Sub-segmental pulmonary embolism - understanding the clinical significance

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Background: The increasing availability of imaging tests with high sensitivity and specificity for the diagnosis of pulmonary embolism (PE) such as Computed Tomographic Pulmonary Angiography (CTPA) and Ventilation Perfusion Single Photon Emission Computed Tomography (V/Q SPECT) has led to a surge in the diagnosis sub-segmental PE (SSPE) raising concern their clinical significance. V/Q SPECT is an acceptable alternative especially when radiation and renal function are of concern.

Aims: Characterize SSPE detected by V/Q SPECT, their clinical presentation, management and outcome.

Methods: A retrospective review of all V/Q SPECT scans for the diagnosis of SSPE was carried out during 2013 and 2014. Patient records of positive V/Q SPECT scans for SSPE were reviewed for presenting symptoms, presence of deep vein thrombosis (DVT), risk factors, provoking factors, treatment and follow-up.

Results: A total of 71 SSPE were identified, 52 female, 19 male, mean age of 61,8 (range 21–93). Of the identified SSPE 83.0% (n=59) presented symptoms of PE. Of the detected SSPEs 69.0% (n=49) were considered to have suffered an idiopathic event. Deep vein thrombosis was documented in only 4 patients. Parenchymal lung disease was detected in 22.5% (n=16). Anticoagulation was initiated in 78.9% (n=56), 30.9% (n=22) were considered for life-long anticoagulation, of the remaining mean duration of anticoagulation was 5.6 months. At 3 months 39.4% (n=28) stated improvement of index symptoms. After a mean follow-up of 22 months, the rate of symptomatic recurrences was 5.7% (n=4), mortality rate of 5.7% (n=4) and only 1 case of major bleeding was observed.

Conclusions: Lack of validated prospective data regarding the treatment of SSPE continues to favor anticoagulation in clinical practice (78.9%). Our data showed a low rate of symptomatic recurrences (5.7%) and bleeding events (1%), this could be in favor of a trial of anticoagulation in symptomatic patients with low bleeding risk. Further and larger prospective studies are needed.

PRE11

Retrievable inferior vena cava filters use in elderly patients

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Background: The safety and efficacy of inferior vena cava (IVC) filter placement for pulmonary embolism prevention in elderly patients have not been well characterized.

Aims: To review indications, complications and follow-up data of elderly patients undergoing IVC filter placement.

Methods: A retrospective review of consecutive admitted patients who underwent IVC filter insertion at a large university hospital with a level I trauma center. Significance between groups was assessed using the chi-square, Fisher′s exact and Mann-Whitney U tests. Overall survival and time to complication analysis was determined using the Kaplan-Meier method.

Results: Overall, 455 retrievable filters were inserted between 2009 and 2014. One hundred and thirty three patients (29.2%) were ≥70 years old. Elderly patients were less likely to have their filter retrieved compared to non-elderly patients (5.3% vs. 21.4%, $P<0.001$). Filter-related complications occurred in 13% of non-elderly patients and 14.3% of elderly patients (P=0.72), most of them occurring in the first 3 months after filter placement (Figure 1). Survival among elderly patients with no evidence of active malignancy was similar to non-elderly patients with a 1-year survival rate of 76.3% vs. 82% in non-elderly (P=0.22) and 2-year survival rate of 73.1% vs. 78.6% in non-elderly patients (P=0.27). Although decreased, survival rates among elderly patients with active cancer were still substantial with a 1-year survival rate of 45% and 2-year survival rate of 40% (Figure 2).

Conclusions: Elderly patients had significantly lower rates of filter retrieval with similar complication rate. Survival rates among elderly patients were substantial and in elderly patients with no active cancer were even comparable to non-elderly patients. When feasible, filter retrieval should be attempted in all elderly patients in order to prevent filter-related complications.

PRE12

Air pollution and venous thrombosis: a meta-analysis

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Background: Exposure to air pollution has been linked to cardiovascular and respiratory disorders, but its effect on venous thrombotic disorders is uncertain.

Aims: We performed a meta-analysis to assess the association between air pollution and venous thrombosis.
Methods: Pubmed, Embase, EBM Reviews, Healthstar, Global Health, Nursing Database, and Web of Science were searched for citations on air pollutants (carbon monoxide, sulphur dioxide, nitrogen dioxide, ozone, and particulate matters) and venous thrombosis. Using a random-effects model, overall risk estimates were derived for each increment of 10 μg/m3 in pollutant concentration. This study is registered in PROSPERO with register number of CRD42014015301.
Results: Of the 484 in-depth reviewed studies, 8 citations involving approximately 100 000 events fulfilled the inclusion criteria. All the main air pollutants analyzed were not associated with an increase in venous thrombosis risk (OR = 1.005, 95% CI = 0.998–1.012 for PM2.5; OR = 0.995, 95% CI = 0.984–1.007 for PM10; OR = 1.006, 95% CI = 0.994–1.019 for NO2). Additional subgroup analyses based on exposure period and thrombosis location provided results comparable with those of the overall analyses. There was no evidence of publication bias.
Conclusions: Studies published so far are not conclusive on the possible role of air pollution as risk factor for venous thrombosis in general population.

PRE13

Acute venous thromboembolism in clinical practice - baseline bleeding risk results from the European PREFER in VTE registry

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Background: Venous thromboembolism (VTE) is a significant cause of morbidity and mortality in Europe. Real-world data on VTE in Europe requires additional research as clinical trials are not representative of all VTE patients, particularly with respect to bleeding risk.
Aims: PREFER in VTE was an international, non-interventional disease registry which aimed to recruit consecutive patients with acute VTE in primary and secondary care in seven European countries and to investigate the bleeding risk.
Methods: PREFER in VTE collected data in a large unselected population, with baseline data on patient characteristics, prevalence of risk factors, and the risk of bleeding. HAS-BLED scores have been shown to be predictive of bleeding in VTE patients.
Results: Of the 3464 patients documented in the database, 3455 qualified for the baseline analysis set (mean age 60.8±16.97 years, 53.0% male). The majority of patients were assessed in hospital (n=2712/3453, 78.5%). The most common VTE-related risk factor was a history of VTE (n=812/3453, 23.5%), followed by varicose veins (n=699/3453, 20.2%), prolonged immobilisation (n=575/3453, 16.7%) and history of cancer (n=552/3451, 16.0%). HAS-BLED scores were available for 3292/3455 patients (95.3%; DVT: n=1932/2056, 94.0%; PE: n=1360/1399, 97.2%). At baseline, bleeding risk was low in the majority of patients, 1864/3292 (56.6%; DVT: n=1147/1932, 59.4%; PE: n=717/1360, 52.7%) patients had a HAS-BLED score 0 or 1, 960/3292 patients (29.2%; DVT: n=530/1932, 27.4%; PE: n=430/1360, 31.6%) had a medium bleeding risk (HAS-BLED score=2) and 468/3292 patients (14.2%; DVT: n=255/1932, 13.2%; PE: n=213/1360, 15.7%) had a high bleeding risk (HAS-BLED score >2. See Figure.
Conclusions: VTE patients at medium-to-high risk of bleeding made up >40% of the cohort. This large unselected European cohort of acute VTE patients shows that being medium to high risk for bleeding is common.

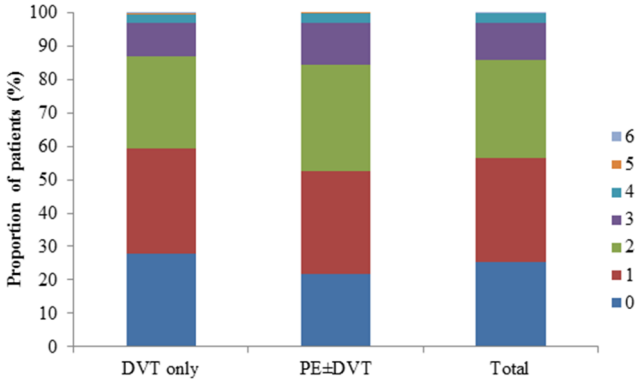

Figure Bleeding risk (HAS-BLED score).

PRE14

Ten years of cerebral venous thrombosis (CVT) in Northeast Melbourne, Australia: male gender and presence of myeloproliferative neoplasm is associated with thrombotic recurrence in unprovoked events

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Background: Cerebral venous thrombosis (CVT) is a rare venous thrombotic event associated with significant morbidity and mortality.
Aims: We review our local experience in the management of CVT compared to other venous thromboembolism (VTE).
Methods: Retrospective evaluation of CVT presentations, from January 2005 to December 2014, at Austin and Northern Health, Melbourne.
Results: 52 patients (30 female, 22 male) with a median age of 40 (18–83) years, presented with 53 episodes of CVT. 17 patients (32%) failed to be diagnosed on initial presentation with a false negative rate of 35% on initial CT brain; all of which were subsequently diagnosed on MRI or CT angiography/venography. 29 episodes (55%) were associated with an underlying risk factor, particularly in females (RR 2.10; p=0.02) compared to men, with hormonal risk factors being most common. The median duration of anticoagulation was 6 months with 11 receiving life-long anticoagulation. 81% had residual thrombosis on repeat imaging, which was not associated with recurrence at the same or distant site. At last follow-up, 2 patients (5%) had a deterioration of modified Rankin score of ≥ 2 from baseline, however, 15 (37%) continued to report ongoing symptoms. Nine (17%) had CVT-related haemorrhagic transformation with 2 resultant CVT-related deaths (RR 22.5; p=0.04). No patients with clearly provoking factors had recurrent VTE. Males with unprovoked events had a higher risk of VTE recurrence (RR 18.2, p=0.05) with all three men who had recurrent VTE being subsequently diagnosed with myeloproliferative neoplasm (MPN). Compared to the VTE population, CVT patients were

younger, had similar rate of provoked events and VTE recurrence, although with significantly higher rate of MPN diagnosis (RR 8.57 (2.11–34.89); p=0.003).
Conclusions: CVT is a rare condition with heterogenous presentations. All recurrences occurred in male patients with subsequent MPN diagnoses, suggesting the need for further evaluation and investigation in males with unprovoked events.

PRE15

Performance of the STA®-Liatest® D-DI plus for the exclusion of pulmonary embolism in emergency departments using an age-adjusted cut-off

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Background: D-dimer measurement is an important step in the diagnosis strategy to exclude pulmonary embolism (PE) when the clinical probability is low to intermediate. Highly sensitive assays are therefore required. D-dimer level increasing with age, the use of an age-adjusted cut-off was recently validated. As the available commercial assays are poorly standardized, each test should be appropriately validated before using it for clinical purpose.
Aims: The clinical performance of the STA®-Liatest® D-DI Plus for the exclusion of PE was thus retrospectively evaluated on the plasmas of patients included in the ADJUST-PE study.
Methods: All consecutive patients who presented to the emergency department of our center with clinically suspected PE were assessed by a sequential diagnostic strategy based on the clinical probability evaluated by the simplified, revised Geneva score. D-dimer measurement was realized in cases of non-high clinical probability with the Vidas® D-Dimer Exclusion™ II assay and the age-adjusted cut-off was applied (ADJUST PE study, NCT01134068). The plasmas were kept frozen and D-dimers were secondly measured with the STA®-Liatest® D-DI Plus assay (provided by Diagnostica Stago).
Results: Over 2 years, D-dimers were assessed in 147 patients with a non-high clinical probability of PE and the global prevalence of PE was 17.7%. Seventy patients (48%) were aged more than 50 years. D-dimer measurement with STA®-Liatest® D-DI Plus assay would have prevented 85 patients (57.4%) from imaging. Using the age-adjusted cut-off maintained the interest of D–dimer measurement in PE exclusion in patients older than 75 yo, as PE would have been excluded in 54% of them (14 out of 26). However, a 86 year-old patient would have been falsely excluded (D-dimers 858 ng/mL), inducing a negative predictive value of STA®-Liatest® D-DI Plus assay of 98.8% [95% IC, 96.5;100].
Conclusions: The STA®-Liatest® D-DI Plus assay seems to be accurate for PE diagnostic strategy in out-patients when using an age-adjusted cut-off.

PRE16

D-dimer assay methods state-of-the art from external quality assessment results

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Background: D-Dimer assessment is a main biological test for the diagnosis strategy of vein thrombosis in ambulatory patient. Thus, analysis methods, which use different assay-types and antibodies, are expected to fulfil analytical performance.
Aims: This work presents D-Dimer main assay methods state-of-the-art from external quality assessment (EQA) results obtained from ProBioQual (PBQ), a French proficiency testing association.
Methods: Participating laboratories (678 in 2015) received, per year, 12 human citrated plasmas lyophilized specimens at 4 different concentration levels. Statistical evaluation was performed according to the ISO guideline 13528 by applying robust algorithm A to calculate consensus value as mean of peer group (PG) (same reagent and device). The methods imprecision is quantified by the inter-laboratories coefficient of variation (CV), and the inaccuracy is evaluated as median (bias50) and 90th percentile (bias90) bias of laboratory results from PG consensus value.
Results: The D-Dimer concentration varies from one to four times depending on the reagent type, showing the absence of standardization for D-Dimer test. While ELISA method CV were about 6%, whatever was the plasma level, turbidimetric methods CV vary from 4% to 16% and other methods CV from 10% to 18%, depending on concentration level. Global bias50 were 7% for plasma under threshold, 5% for plasma near threshold, and < 4% for D-Dimer high level. Global bias90 were, respectively, about 21%, 14% and 10%. Bias assessment according to each assay-type shows that bias90 are directly dependent on plasma level for all methods, except for ELISA.
Conclusions: In this work we show that methods imprecision depends on assay-type and also on plasma level. Since acceptable limits of inaccuracy are usually determined according to bias90, they should be assessed as function of plasma level.

PRE17

Impact of severe ADAMTS-13 deficiency in patients with thrombotic thrombocytopenic purpura (TTP) on recurrences

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Background: Several studies are evaluating in TTP patients the utility of serial measurements of ADAMTS13 in predicting the clinical course during initial therapeutic approach and early relapse.

Aims: To evaluate, in patients with TTP, ADAMTS-13 activity and its inhibitors at diagnosis and to establish the prognostic value of these markers for recurrences.
Methods: Blood samples were collected from 54 TTP patients: 48 idiopathic, 3 drug-induced and 3 with bone marrow transplantation (BMT)-associated TTP. ADAMTS-13 activity, inhibitors, and anti-ADAMTS-13 antibodies were measured in plasma by commercial kit.
Results: A complete ADAMTS-13 activity deficiency (i.e. < 5%) was detected in patients at the onset of TTP. In 83% of patients, the deficiency was associated with an inhibitory activity and high anti-ADAMTS-13 antibody titer. At remission, 19 of them showed normal ADAMTS-13 levels. (i.e. >50%), while 5 had moderate (i.e. 21–50%), 4 severe (i.e. 5–20%), and 5 complete (i.e. < 5%) deficiency. In the majority of cases, the normalization or the partial recovery of ADAMTS-13 was associated to the reduction of the antibody levels. Nine of the 10 patients with ADAMTS-13 activity ≤ 20% during remission had at least one relapse, as compared to only 9 out of the 21 patients with ADAMTS-13 activity >20% ($P = 0.004$). Four patients showed severe ADAMTS-13 activity deficiency associated to the absence of inhibitory activity and anti-ADAMTS13 antibodies: these patients were identified as carriers of mutations in ADAMTS13 gene. BMT-associated TTP had moderate deficiency of ADAMTS-13 activity and no anti-ADAMTS-13 antibodies. Differently, patients with drug-associated TTP had severe ADAMTS-13 deficiency with anti-ADAMTS-13 antibody positivity.
Conclusions: Our data suggest that in autoimmune TTP patients, ADAMTS-13 lower than ≤ 20% may be a useful marker for risk stratification and identification of patients at high risk of recurrences.

PRE18
Description of a new prothrombin gene variant C20214T

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Background: G20210A (rs1799963; NM_000506.3 c.*97G>A) prothrombin gene variant, first described by Poort *et al* (Blood, 1996), has been associated with an increased risk of venous thromboembolism (VTE). Since this description, other rare polymorphisms of *F2* gene (encoding prothrombin) have been reported. However, their frequency rate in general population and their potential association with VTE remain uncertain.
Aims: Here we described a new *F2* variant in a 23-year old woman sent by her gynecologist for a thrombophilia check-up before changing her oestroprogestative oral contraception. This patient had a familial but not a personal history of VTE.
Methods: Thrombophilia check-up included standard coagulation analysis, *F2* and *F5* gene polymorphism determination by melting curve analysis after real-time PCR on a capillary-based Lightcycler®, finally sequencing of *F2* gene 3'UTR region encompassing 20210 position using a ABI 3130® capillary sequencer.
Results: Coagulation factors were in normal ranges, in particular factor II and inhibitors. *F5* genotyping for G1691A transition revealed a wild-type genotype. By contrast, melting curve analysis for G20210A polymorphism showed an unusual curve progression. While the melting points™ corresponding to the wild-type and the 20210 A allele were defined by the manufacturer to be at respectively 59°C (56.5–62) and 49°C (46.5–51.5), patient DNA analysis showed a Tm at 59.5°C and 53.5°C (Figure 1). Sequencing revealed a C>T heterozygous transition at position 20214, 4 bp downstream of G20210 A *F2* variant in the 3' UTR (NM_000506.3 c.*101C>T).This variant was not found either in Exome Variant Server or 1000 genomes.

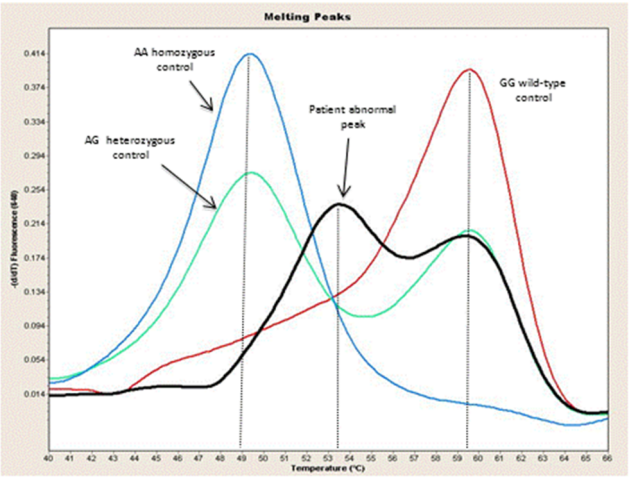

Figure 1 Fusion curve profile on Lightcycler.

Conclusions: To conclude, this new C20214T *F2* variant was not associated in our patient with any personal history of thrombosis, but with familial VTE. We had characterized it because of the methods used (Lightcycler and sequencing). By contrast, other diagnostic methods (RFLP, GeneXpert® quantitative PCR) would have failed in its detection.

PRE19
Venous Thromboembolism and Variants of *FGG*, *F11* and AB0 in Czech Republic

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Background: Venous thromboembolism (VTE) is also hereditary dependent.
Aims: The aim was to assessed the frequencies of risk alleles of FGG, F11 and AB0 genes in healthy persons and in VTE patients in Czech Republic and determine their level of VTE risk (odds ratio, OR).
Methods: Mutations of fibrinogen gamma gene *FGG* (rs 6536024, rs 2066865), *F11* gene (rs 4253399, rs 2289252 and rs 2036914) and in AB0 blood group (rs 579459, rs 2519093 and rs 8176719) were tested in age matched groups of patients with VTE (n 570, 0.65 female) and healthy persons (n 570, 0.43 female) from the area of Prague city and Middle Bohemia region. The polymorphisms were determined using PCR by FRET process with LightCycler® 480 System (Roche Diagnostics, Prague, CZ). Specific primers and fluorescently labelled probes were designed in cooperation with TIB MOLBIOL (Berlin, Germany). Fisher extract test was determined and OR > 1.5 was considered as clinically significant. The study was performed in scope of the project "RVO VFN 64165" which has been approved by local ethics committee.
Results: Frequencies of risk alleles in the VTE patients group and in the control group were: 0.586 and 0.498 for allele C of *FGG* rs 6536024 (OR 1.4252; $p < 0.001$), 0.313 and 0.254 for allele T of *FGG* rs 2066865 (OR 1.3364; $p = 0.0019$), 0.387 and 0.450 for allele G of *F11* rs 4253399 (OR 1.2968; $p = 0.0022$), 0.388 and 0.468 for allele T of *F11* rs 2289252 (OR 1.3916; $p < 0.001$), 0.522 and 0.594 for allele C of

F11 rs 2036914 (OR 1.3384; p = 0.0006), 0.262 and 0.336 for allele C of AB0 rs 579459 (OR 1.4064; p < 0.001), 0.266 and 0.336 for allele T of AB0 rs 495828 (OR 1.3946; p < 0.001), 0.030 and 0.031 for allele T of AB0 rs 2519093 (OR 1.0026; p = 0.9920) and 0.445 and 0.547 for allele G of AB0 rs 8176719 (OR 1.5098; p < 0.001).

Conclusions: Only polymorphism of non 0 blood group (rs 8176719) determined of the VTE risk with OR above 1.5 in this study. We also confirmed the importance of *FGG rs 6536024* and *F11 rs 2289252* for VTE (both with p < 0.001).

PRE20

High sensitive LOCI based F 1 + 2 immunoassay for quantification of prothrombin fragment 1 + 2 in human plasma samples on the new INNOVANCE BCS LT system

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Background: We describe the development and initial analytical performance of a homogeneous sandwich immunoassay (INNOVANCE® LOCI F 1 + 2 Reagent Cartridge)* for measurement of prothrombin fragment 1 + 2 using LOCI® technology on the new INNOVANCE BCS® LT System* from Siemens Healthcare.

Aims: Characterization of the LOCI based F 1 + 2 immunoassay.*:

Methods: LOCI technology is a homogeneous, chemiluminescent immunoassay approach that consists of a streptavidin-coated Sensibead particle, a Chemibead particle with immobilized antibody, and a second biotinylated antibody. In the assay, the analyte forms bead-aggregated immune complexes via a primary neo-epitope-specific monoclonal antibody and an immune-complex-specific secondary monoclonal antibody, thereby generating a signal chain leading to a delayed, analyte-concentration-dependent luminescence emission.

Results: The INNOVANCE LOCI F 1 + 2 Reagent Cartridge* is an automated, high sensitive immunoassay on the new INNOVANCE BCS LT System*. The assay was characterized regarding precision, limit of quantitation, stability, reproducibility, linearity and interference. The assay consists of four components: liquid LOCI reagents available in a light-protective reagent cartridge, a lyophilized calibrator, lyophilized controls, and a special diluent used for the automatic dilution of the calibration curve. The system enables long-term onboard reagent stability by using evaporation protection and reagent cooling.

Conclusions: We conclude that use of LOCI technology on the INNOVANCE BCS LT System* has the potential to provide excellent sensitivity and precision suitable to reliably measure F 1 + 2 in human citrated plasma in a broad range without sample dilution and with a fast turnaround time.

* Under development. Not available for sale. Product availability varies by country. BCS, INNOVANCE, LOCI, and all associated marks are trademarks of Siemens Healthcare Diagnostics Inc., or its affiliates. A91DX-HHS-151834-GC1-4A00

PRE21

Getting the right product to the right patient - proposed platelet concentrate screening to improve transfusion outcome

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Background: Ten to 20% of platelet transfusion patients become refractory, defined by two transfusions in a row resulting in corrected count increments (CCI) under $5x10^9$/L. Only 3-5% of refractoriness is caused by antibody mismatch. The remainder may be caused by an unidentified mismatch between platelet concentrate (PC) and recipient. Our clinical study suggests a greater risk of refractoriness if a patients receives heterogeneous PC while febrile.

Aims: Assess the potential of dynamic light scattering (DLS) for improving product-to-patient matching for PC transfusion.

Methods: Two hundred hemato-oncology patients were enrolled with informed consent in a prospective 2-arm randomized controlled trial. ThromboLUX (TLX), a DLS device, was used to screen the PC prior to transfusion. A heterogeneous-homogenous threshold was set at a TLX score of 10. The experimental group (Exp) received PC with TLX score ≥ 10, the control group (Ctl) received PC of any score. One hr and 24 hr CCIs were monitored. The TLX score was tested as a predictor of 24 hr CCI. Data was validated and analyzed by two groups of biostatisticians.

Results: The 1 hr and 24 hr CCIs had a weak correlation (R^2=0.50). Exp had a higher 24 hr CCI than Ctl, without statistical significance (P = 0.11). Table 1 shows a surprisingly high rate of refractoriness. Table 2 shows the effect of heterogeneous transfusions in combination with refractoriness on length of stay and number of transfusions. Patients who became refractory had a significant elevation in body temperature (0.74°C, P < 0.001) at transfusion.

Table 1

	Total	Refractory	Non-Refractory	% Refractory
At least one heterogeneous transfusion	**93**	**34**	**59**	**37%**
No heterogeneous transfusion	69	11	58	16%

Table 2

	Length of hospital stay in Days	Number of platelet transfusions
Group1: At least one hetero transfusion & refractory Mean(SD) (N = 34)	29.9 (11.4)	13.6 (10.7)
Group2: At least one hetero transfusion and non-refractory Mean(SD) (N = 59)	22 (9.9)	4.6 (3.3)
Group3: No hetero transfusion and refractory Mean(SD) (N = 11)	20.1 (4.5)	6.3 (3.3)
Group4: No hetero transfusion and non-refractory Mean(SD) (N = 58)	17.8 (8.6)	2.2 (1.6)
P-value 1V3 (T-test)	0.0002	0.0011
P-value 1V4 (T-test)	<0.0001	<0.0001

Conclusions: The weak correlation of 1 hr and 24 hr CCIs suggests patient factors interfere with 24 hr CCI predictions. Thus, the patient plays a role in transfusion effectiveness. The elevation in refractory patient body temperature points to one such patient factor. Heterogeneous transfusions seem to increases the chances of refractoriness leading to longer hospital stay and more transfusions. It is possible that febrile patients should not receive heterogeneous PC.

PRE22

Evaluation of the risk factors of recurrence of cerebral venous thrombosis

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Background: Cerebral vein thrombosis (CVT) is a vascular disease with unclear incidence due to its rarity and scanty epidemiological studies. The investigation of risk factors in individuals with recurrent VTE after a first CVT episode will provide better targeting of groups of patients who may benefit from extended anticoagulation therapy.
Aims: Evaluate the risk factors related to venous thromboembolism recurrence after a first CVT episode.
Methods: This prospective study involved patients with a first episode of diagnosed CVT from four brazilian centers: Clínica Hematológica; Hospital das Clínicas da Universidade Federal de Minas Gerais; Hemocentro da Universidade Estadual de Campinas; Serviço de Hematologia da Universidade Federal de São Paulo. Eligible patients were enrolled in the study between 04/01/2000 and 06/30/2014 by convenience sample.Inclusion criteria were 18 years of age or older with an objectively diagnosed CTV as a first confirmed thromboembolic episode.End point was VTE recurrence in any other venous site. The incidence rate of recurrent thrombotic events was measured as the number of events over time of accumulated observation.
Results: Two hundred and three patients, mean age 30.8 years, had a follow-up duration of 3.0 years and most patients were women. Among female patients of reproductive age, 106 developed CVT during oral contraceptive use, pregnancy or puerperium. In this study, patients had a VTE recurrence rate of 1.6 cases per 100 patients-year considering all participant centers. The incidence of recurrent venous thromboembolism was higher in male patients vs. female and patients with positive Factor V Leiden.Among the recurrent cases presented VTE secondary to triggering factor/precipitating factor.
Conclusions: The recurrence rate of VTE after a first CVT event, although low, suggests a higher prevalence in male patients. Other studies are needed to assess the association of the risk factors of VTE recurrence after a first CVT event.

PRE23

Does thrombin generation test predict the outcome of ischemic stroke after thrombolysis treatment?

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Background: Today, thrombolysis by recombinant tissue plasminogen activator (rtPA) is the most effective therapy in acute ischemic stroke (IS).
Aims: Our aim was to find out whether results thrombin generation test (TGT), a global hemostasis test, might predict thrombolysis outcomes.
Methods: Study population included 120 consecutive acute IS patients, all within 4.5 h of symptom onset, who underwent thrombolysis by rtPA. Blood samples were taken before therapy and TGT was performed. Clinical data of patients using the National Institutes of Health Stroke Scale (NIHSS) score were registered on admission, day 1 and 7 after therapy. CT images on admission and day 1 were analyzed and the ASPECT score was assessed. Intracranial hemorrhage was classified according to the ECASS II criteria. Long term functional outcome was defined at 3 months after the event by the modified Rankin scale. All patients gave informed consent.
Results: Endogen thrombin potential (ETP) and peak thrombin were significantly higher in patients with atherothrombotic IS. Among the TGT parameters, only time-to-peak was associated with stroke severity. No correlation was found between TGT parameters and the ASPECT scores. An ETP result in the lower quartile was associated with an increased risk for therapy-associated symptomatic intracranial hemorrhage (SICH) (OR:17.5; 95%CI:1.5-212.7, $P < 0.05$). Peak thrombin result in the lower quartile was also significantly associated with SICH (OR:15.1; 95%CI:1.4-166.0, $P < 0.05$). Low values of ETP but not those of peak thrombin were associated with mortality. A multiple logistic regression model revealed that an ETP result in the lower quartile is an independent predictor of mortality within the first 2 weeks (OR:5.52; 95%CI:1.5-20.1, $P < 0.01$) and 3 months after the event (OR:3.9; 95%CI:1.3-12.0, $P < 0.05$).
Conclusions: Low levels of ETP and peak thrombin parameters increase the risk of SICH. Low values of ETP is an independent predictor of mortality after thrombolytic treatment.
Funding: OTKAPD111929.

PRE24

An automated indicator for interfering substances in hemostasis

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Background: Hemoglobin, bilirubin, and triglycerides are known to interfere with many hemostasis assays. The current laboratory standard for detection of interfering substances is the visual screening of each sample. The INNOVANCE® BCS® LT System* employs a dedicated module for the detection of hemoglobin (H), bilirubin/icterus (I), and turbidity/lipemia (L).
Aims: Comparison of the performance of the HIL module of the INNOVANCE BCS LT System* to visual inspection of samples.
Methods: The HIL module consists of a capillary measurement cell with a photometric unit. The module reports the HIL assessments in index levels from 1 (low) to 9 (high). Excess amounts of frozen patient samples were used at two external sites[2,3] to compare the visual impression with the result obtained by the HIL module. In cases of doubt, each sample was also assessed by a clinical analyzer.
Results: The results differ depending on the interfering substances.
For hemoglobin, the correlation between the visual impression and the HIL module was good, with a slight overestimation compared to the visual impression.
For bilirubin, the results of the HIL module correlated well to the clinical analyzer, while visual discrimination among the index levels seemed to be difficult.
Turbidity caused by lipemia is very heterogeneous, as the lipoproteins' VLDL and chylomicrons vary in size, and the observed differences

compared to the visual classification revealed an underestimation by the HIL module.

Conclusions: This automated method improves consistency and reproducibility compared to a visual check of samples for HIL and is less susceptible to human bias as demonstrated in the case of bilirubin.

*Product under development. Not available for sale. Product availability may vary from country to country and is subject to varying regulatory requirements

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PRE25

Establishing reference intervals for technothrombin®TGA based on plasma samples from healthy blood donors

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Background: Technothrombin® TGA (TGA, Technoclone, Austria) is a valuable tool for investigating blood coagulation activity, regarding elevated thrombin activity – thrombotic tendency – or depressed activity – bleeding tendency – under the stimulation by reagent Low (RCL) and High (RCH) containing different concentrations of tissue factor and phospholipids (Technoclone, Austria).

Aims: This TGA method is based on a fluorogenic automated standardized procedure using Ceveron®alpha TGA analyzer and software (Technoclone, Austria). Parameters calculated for thrombin generations (TGA): Lag Phase, Time to Peak, Peak Thrombin (Peak, nM) and Area under the Curve (AUC, nM).

Methods: To establish reference intervals for Peak and AUC plasma samples of 100 healthy blood donors (50 male, age, 37.7 ± 12.4; 50 female, age, 42.9 ± 11.5) were investigated. After vein puncture blood was collected in Vacuette citrate coagulation tubes (Greiner, Austria) and PPP was prepared by centrifugation of the tubes according to the recommendations of the manufacture. Than plasma was immediately stored at −70°C for further coagulation tests. TGA was measured on Ceveron analyzer using RCL and RCH reagent and was repeated after removal of microparticles from plasma (MFP).

Results: For Peak and AUC 25% percentile, median, 75% percentile, mean and STD were calculated for all samples, as well as separated for female and male. Reference intervals are dependent on the used trigger as well as on the sample material PPP or MFP and they differ also between male and female.

Table TGA reference ranges.

		All	Female all	Male all		All	Female all	Male all
Peak Thrombin PPP RC Low	25% Percentile	87.1	95.5	79.6	Peak Thrombin MFP RC Low	52.7	63.8	48.1
	Median	110.0	129.0	99.1		71.4	86.1	63.2
	75% Percentile	164.3	204.6	143.5		114.3	123.9	97.6
	Maximum	367.6	367.6	332.5		215.7	210.4	215.7
	Mean	136.4	151.2	121.2		87.7	96.4	78.9
	Std. Deviation	68.0	71.5	61.4		45.7	42.9	47.2
Peak Thrombin PPP RC High	25% Percentile	169.1	183.3	157.6	Peak Thrombin MFP RC High	150.9	151.4	147.1
	Median	212.4	227.9	199.4		193.6	206.5	182.8
	75% Percentile	336.1	381.4	305.3		282.7	317.6	274.6
	Maximum	626.3	600.7	626.3		657.8	657.8	566.4
	Mean	262.5	277.0	247.4		237.9	252.2	223.0
	Std. Deviation	130.6	130.8	129.9		127.1	133.8	119.3

For age groups greater than or < 40 years differences in reference intervals are also observed.

Conclusions: We recommend that reference intervals should be used separately for men and women and probably also in an age-dependent manner. The reference intervals should also be tested under a variety of clinical issues on their operational capability to distinguish pathological from

PRE26

Sudden onset vision loss as an initial manifestation of elevated serum lipoprotein(a) levels

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Background: Isolated cilioretinal artery occlusion (CLRAO) or cilioretinal venous occlusion (CRVO) may cause sudden loss of vision. In such cases, prothrombotic risk factors should be investigated. Lipoprotein(a) [Lp(a)] is an atherogenic lipoprotein particle which displays adjunctive thrombotic properties by inhibition of the fibrinolytic pathway.

Aims: In this study, we reported two cases with retinal vascular occlusion associated with elevated serum Lp(a) levels.

Methods: Case 1 concerns a 13-year-old female with a complaint of sudden vision loss in the right eye. She was diagnosed of having right CLRAO. Thrombophilia work-up was normal except the high plasma Lp(a) level of 373.3 mg/dl. In order to reduce Lp(a) levels, cascade filtration procedure was performed and the patient was started on oral niacin treatment. On the eighth day the visual acuity had fully recovered. Cascade filtration was applied once every 2 week. We aimed to keep the Lp(a) levels below 100 mg/dl. She is ongoing in good condition with reduced levels of Lp(a).

Results: Case 2 concerns a 17-year-old female with a complaint of sudden vision loss in the right eye. Ophthalmologic examination revealed that she was having combined CRVO and CLRAO. Laboratory parameters were remarkable for an elevated level of Lp(a) of 94.8 mg/dl. Thrombophilia work-up was normal. Treatment with anticoagulants and oral niacin were initiated. The patient's visual acuity gradually improved but she developed optic atrophy. Cascade filtration was not applied because spontaneous recanalization was developed. Oral niacin treatment was started.

Conclusions: Elevated plasma Lp(a) levels might be related with retinal vascular occlusions and should be checked in such cases both to determine the etiology of the vascular occlusion. Oral niacin treatment and cascade filtration may be benefit for these patients. There is no definitive cut off level of Lp(a) for cascade filtration. Further studies are needed to optimize to perform cascade filtration for children.

PRE27

Concerns on reliability of haemostasis activation markers in a cohort of healthy volunteers

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Background: Recent data have analyzed the fundamental coupling between coagulation and innate immunity, indicating the central role of neutrophil-derived circulating nucleosomes and free DNA on the promotion of coagulation. Elevated levels of circulating nucleosomes were recently associated with deep vein thrombosis and proposed to be helpful for treatment of patients. However, no reliability data are available and there is no comparison with conventional markers of haemostasis activation such as D-Dimers.

Aims: Reliability study of nucleosomes, free DNA and D-Dimers in healthy controls.
Methods: We recruited healthy controls to be tested every 4 weeks for 6 months. Quantification of plasma nucleosomes was performed by ELISA, following the recommendations described by Holdenrieder S. (2001). Plasma free DNA was quantified by Q-PCR assay. D-dimers were assayed using the gold standard method (ELFA, bioMérieux). The reliability of measurements was evaluated by computing the intraclass correlation coefficient (ICC), finally qualified as being good (ICC $\geq$ 0.9), acceptable ($\geq$ 0.8), moderate ($\geq$ 0.6) and poor (< 0.6). ClinicalTrials.Gov ID: NCT01559207.
Results: 15 healthy controls accepted to be followed monthly during 6 months after the inclusion visit, thus corresponding to 7 blood samples per patient. The ICC values for free DNA, nucleosomes, and D-Dimers were 0.091 [CI 95%: 0.026-0.328], 0,538 [CI 95%: 0.334-0.764], and 0.807 [CI 95%: 0.666-0.917] respectively, suggesting reliabilities ranging from poor to acceptable.
Conclusions: Our study shows that biological parameters significantly vary over a short time frame in normal individuals. The clinical value of such intra-individual normal variations is currently unknown. Despite largely static investigation habits, studying the intrinsic clinical value of individual patterns of markers' variability is warranted.

PRE28

Concerns on reliability of haemostasis activation markers in patients with a history of venous thromboembolism

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Background: Recent data have analyzed the fundamental coupling between coagulation and innate immunity, indicating the central role of neutrophil-derived circulating nucleosomes and free DNA on the promotion of coagulation. Elevated levels of circulating nucleosomes were recently associated with deep vein thrombosis and proposed to be helpful for treatment of patients. However, no reliability data are available and there is no comparison with conventional markers of haemostasis activation such as D-Dimers.
Aims: Reliability study of nucleosomes, free DNA and D-Dimers in patients with a history of venous thromboembolism.
Methods: We recruited patients with a venous thromboembolism (VTE) history to be tested every 4 weeks for 6 months. Plasma nucleosomes were quantified by ELISA, following the recommendations described by Holdenrieder S. (2001). Plasma free DNA was quantified by Q-PCR assay. D-dimers were assayed using the gold standard method (ELFA, bioMérieux). The reliability of measurements was evaluated by computing the intraclass correlation coefficient (ICC), finally qualified as being good (ICC $\geq$ 0.9), acceptable ($\geq$ 0.8), moderate ($\geq$ 0.6) and poor (< 0.6). ClinicalTrials.Gov ID: NCT01559207.
Results: 15 patients accepted to be followed monthly during 6 months (7 blood samples per patient). The ICC values for free DNA, nucleosomes, and D-Dimers were 0.161 [CI 95%: 0.008–0.398], 0,213 [CI 95%: 0.042–0.463], and 0.639 [CI 95%: 0.462-0.917] respectively, suggesting reliabilities ranging from poor to low.
Conclusions: Although hundreds of articles exist describing markers of haemostasis activation, none rigorously assess their natural intrinsic intra-individual behaviour. Our study shows for the first time that biological parameters spontaneously vary over a short time frame in VTE patients. The clinical significance and consequence of such variations, if any, should be investigated.

PRE29

Mean platelet volume as a biomarker of disease activity in polymyalgia rheumatica

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Background: Mean platelet volume (MPV) was reported to be a biomarker for platelet activation. MPV might also be a biomarker of disease activity in rheumatic diseases.
Aims: We evaluated MPV in polymyalgia rheumatica (PMR) patients. We also analyzed their relationship between disease activity.
Methods: 85 PMR (27 males, 58 females, mean age: 68.65 $\pm$ 5.3 years) and 48 healthy controls (20 males, 28 females, mean age: 68.14 $\pm$ 9.9 years) were included. The clinical and laboratory data were obtained from medical charts. Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and MPV were recorded at the time of diagnosis and after 3 month of therapy. All subjects gave informed consent and ethical approval was obtained.
Results: Sex-and-age profiles were similar between groups. MPV in PMR (8.00 $\pm$ 1.06 fL) group was significantly lower than in healthy controls (9.59 $\pm$ 1.47 fL) ($P < 0.001$). Also, ESR, CRP and absolute neutrophil count in PMR group were significantly higher than in healthy controls ($P < 0.001$). While MPV (8,29 $\pm$ 1.12 fL) increased significantly in PMR group ($P = 0.001$) after 3 months of treatment; ESR (from 61.02 $\pm$ 31.04 mm/hr to 23.84 $\pm$ 15.39 mm/hr); CRP (from 5.33 $\pm$ 6.1 mg/dl to 0.69 $\pm$ 0.94 mg/dL); and platelet count (from 316000 $\pm$ 94739/mm^3 to 262582 $\pm$ 66013/mm^3) decreased significantly ($P < 0.001$). Pearson correlation analysis in PMR group revealed that MPV correlated with platelet count ($r = 0.25$, $P = 0.48$).
Conclusions: MPV in PMR group was significantly lower than in healthy controls and it increased significantly after treatment. MPV was a negative inflammatory biomarker for disease activity in PMR patients.

PRE30

Thrombocytopenia and altered coagulation as independent predictors of the etiology of acute undifferentiated febrile illness (AUFI) in adults presenting to emergency in a tertiary care hospital in North India

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Background: Acute Undifferentiated Febrile Illness (AUFI) refers to a febrile illness with no indication of an organ-specific disease or a focal site of infection. The main etiologies of AUFI in the developing world include malaria, dengue, enteric fever, rickettsiosis, leptospirosis, Hantavirus, Japanese encephalitis. Early diagnosis and subsequent treatment can be lifesaving.
Aims: To evaluate predictors of the etiology of AUFI at presentation in emergency.
Methods: 135 adult patients (more than or equal to 14 years of age) with a diagnosis of AUFI (fever (body temperature > 101°F) of 14 days or less in duration without any localized source of infection on initial clinical evaluation) were enrolled.

All these patients with AUFI were evaluated on the basis of a standard proforma and were evaluated for malaria(peripheral smears/rapid diagnostic kits), scrub typhus(PCR/IgM ELISA), leptospirosis(IgM ELISA), enteric fever by blood cultures and dengue by dengue (NS1 antigen test and IgM ELISA).

Results: Among the 135 patients studied, 82 were males The mean age of the patients enrolled in the study was 34.8 ± 15.8 years Out of 135 patients, 8 patients (5.93%) died.

Among the 135 patients, the etiological distribution of AUFI was scrub typhus 54 (40%), malaria 13 (9.63%) dengue fever 2 (1.48%), enteric fever 1 (0.74%), leptospirosis 1 (0.74%). No definite diagnosis could be made in 51 (37.78%) patients. Co-infections included nine (6.67%) cases of scrub typhus with malaria and one (0.74%) case of scrub typhus with dengue.

The variables associated with an increased likelihood of scrub typhus were presence of ARDS hepatomegaly/splenomegaly, elevation of ALP and a deranged aPTT at presentation. On the other hand patients with malaria at presentation had jaundice, severe thrombocytopenia(< 10,000) as compared to other etiologies of AUFI.

Conclusions: Thrombocytopenia and derranged cltting parametrs help in predicting etiology of AUFI.Further studies are needed to test this hypothesis.

PRE32

Red cell distribution width is associated with atherosclerotic cardiovascular mortality in gouty arthritis

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Background: Red cell distribution width (RDW) is a measurement of the heterogeneity in size of the circulating erythrocytes; increased RDW values are associated with poor cardiovascular (CV) outcome in general population and in rheumatoid arthritis (RA).The frequency of CV mortality in gouty arthritis (GA) is increased.

Aims: We evaluated RDW in GA patients and analyzed whether RDW might be related with disease specific parameters.RA patients were the control group.

Methods: Eighty-four GA (65 M,19 F,mean age:64.7 ± 13.4),79 RA (38 M,41 F,mean age: 63.5 ± 11) patients,61 healthy controls (34 M,27 F,mean age:65.4 ± 5.7) were included.The clinical and CV mortality data were obtained from medical charts.Laboratory parameters including RDW were recorded at the time of diagnosis and 1 month after therapy.Informed consent and local ethical committee approval were obtained.

Results: RDW value in GA (14.6 ± 1.4 fL) and RA (15.3 ± 2 fL) groups were significantly higher than in controls (13.5 ± 0.8 fL) (p values< 0.001).RDW value in RA patients was significantly higher than in GA patients ($P = 0.004$).RDW values were re-evaluated after resolution of gout attack in 57 patients and after effective treatment in 66 RA patients.In both groups, RDW increased significantly after treatment (GA:14.5 ± 1.3 vs. 15.2 ± 1.6,RA: 15.3 ± 2.1 vs. 16.7 ± 2.5, p values < 0.001). In both groups, the change in RDW value (RDW before treatment minus RDW after treatment) correlated negatively with changes in ESR(GA: r = -0.45, RA: -0.4, p values=0.002).Eight GA and 11 RA patients died from CV diseases during follow-up (median: 84 months).GA patients who died from CV causes had significantly higher RDW values than others (15.6 ± 1.5 vs. 14.4 ± 1.4, $P = 0.034$).

Conclusions: RDW value was significantly higher in GA and especially RA patients when compared to controls.Interestingly, after resolution of acute inflammation, RDW increased significantly in both groups.CV mortality may be associated with higher RDW values in GA patients.

PRE33

Biomarkers of hypercoagulability and clinical variable related with VTE risk in ambulatory patients with lung adenocarcinoma on chemotherapy. The ROADMAP study

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Background: In ambulatory patients with lung adenocarcinoma (APLA) the risk of VTE increases during chemotherapy (CTx). The identification of clinically relevant biomarkers of hypercoagulability (BH) and clinical variables could lead to better evaluation of VTE risk.

Aims: The prospective longitudinal non interventional study ROADMAP, was designed to identify the most relevant related risk factors of VTE in ambulatory patients with LA on CTx.

Methods: APLA were included and followed up at 3, 6 and 12 months. Documented symptomatic VTE was the end-point of the study. Blood samples were collected at inclusion and assessed for BH (Table). Assays and reagents were from Stago (France). A Clinical Research Form was completed by patients' interview at inclusion. Univariate analysis was done.

Results: The study included 150 patients (mean age 65 years, 73% male). The LA diagnosis was done within 6 months before inclusion in 70% of them and 90% had advanced or metastatic disease at inclusion. In 85% of patients the ECOG performance status was < 3. In 1 year follow up 12 symptomatic VTE episodes occured (8%), 75% of which occurred within the 3 months from inclusion. The univariate analysis identified the odds ratio for each variable as shown in Tables. (*$P < 0.05$)

Table

Biomarkers	Odds ratio (95% CI)
Tissue factor activity (TFa)	0.991 (0.91–1.079)
D-Dimers	0.917 (0.634–1.325)*
PPL-ct	1.058 (1.008–1.144)*
Lag-time	0.603 (0.294–1.235)
MRI	1.016 (1.003–1.032)*
Peak	1.006 (0.997–1.016)
ETP	1
ttPeak	0.522 (0.307–0.887)

Table

Clinical variables	Odds ratio (95% CI)
Recent hospitalization within the last month before assessment	4.274 (0.946–1.333)*
Time since diagnosis of the cancer	4.679 (0.443–29.4)*
Cancer progression	4.114 (1.014–16.692)*

Clinical variablesConclusionsThe Mean Rate index (MRI) and the Procoagulant Phospholipid clotting time (PPL-ct) are the BH related with the risk of VTE in APLA. Their combination with the clinically relevant variables of patients and cancer related VTE risk may build a RAM specific for this group of cancer patients.

PRE34

Increased mortality associated with omission of venous thrombo-embolism (VTE) prophylaxis in a rural intensive care unit in Victoria, Australia

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Background: Omission of venous thrombo-embolism (VTE) prophylaxis in the first 24 h of intensive care admission (ICU) is associated with increased mortality.
Aims: We aim to investigate the outcomes of patients who did not receive VTE prophylaxis in the first 24 h in a rural ICU.
Methods: Retrospective study of patients admitted to ICU at Wimmera Base Hospital, Victoria between January 2013 and December 2014. Data collected included VTE status, Acute Physiology and Chronic Health Evaluation II (APACHE II) score and mortality. Patients in whom VTE prophylaxis was contra-indicated or not indicated were excluded.
Results: Between 2013 and 2014, there were 1345 admissions to ICU. We excluded 486 patients (36%). Eight hundred and forty-seven patients (99%) received VTE prophylaxis within 24 h of ICU admission. Twelve patients (1%) did not receive VTE prophylaxis when indicated. There was an increased mortality rate in patients who did not receive VTE prophylaxis when indicated compared to patients were treated with VTE prophylaxis (17% v 4%). Patients who did not receive VTE prophylaxis who died were observed to have a higher APACHE II score compared to patients who survived and did not have VTE prophylaxis (27 vs 9.6).
Conclusions: This study found omission of VTE prophylaxis in the first 24 h of ICU admission was associated with increased mortality. This is consistent with previous literature. VTE prophylaxis is more imperative in patients with higher acuity of illness, based on APACHE II score.

PRE35

Thromboelastography and Thrombin Generation Test Follow-Up in Patients Developing Sepsis or Systemic Inflammatory Response Syndrome

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Background: Thromboelastography has been mostly used to detect hypocoagulability in different settings. Only few data are available, on the performance of this method to diagnose an hypercoagulable state, especially in sepsis.
Aims: The objective of this study was to test whether thromboelastography (TEG®)-kaolin was able to detect an hypercoagulable state in comparison to thrombin generation test (TGT) and standard coagulation tests in patients with high risk of hypercoagulation, in a surgical setting. The original feature of our study is the selection of participants at high risk of developing an hypercoagulable state with a close follow-up in order to monitor TEG® profile modifications throughout the study period.
Methods: This is a prospective observational pilot study in a university hospital intensive care unit. All consecutive patients who underwent major general surgery at high risk of post-operative systemic inflammatory response syndrome (SIRS) or sepsis were included. The Thromboelastograph Analyser® for TEG®, the Calibrated Automated Thrombogram® system Fluoroskan Ascent Thermo® for TGT and fibrinogen concentration were performed. Patients were followed for at least 7 days with 3–5 samples per patient. Ethical committee approval: 2013-A99107-38.
Results: Fourteen patients were included between March 2014 and October 2015, with 12 SIRS and 9 sepsis. All patients received heparin or LMWH for venous thromboembolism prophylaxis. The relevant parameters Mean±SD values of the first and last day of the follow-up are presented in the table. We found a high relationship between maximal amplitude and fibrinogen concentration. The endogenous thrombin potential and other TGT parameters didn't change.

Table TEG®, TGT and fibrinogen concentration results.

	TEG® Reaction Time(R) (min)	TEG® maximal amplitude (MA) (mm)	TEG® angle(°)	Thrombin generation test Endogenous thrombin potential(ETP) (nM.min)	Fibrinogen (g/L)
Before surgery	5.5 ± 1.3	63.8 ± 5.8	67.8 ± 4.1	1923 ± 344	2.9 ± 0.7
Last day of follow-up	6.8 ± 1.2	72.3 ± 11.5	69.1 ± 7.3	1803 ± 334	6.7 ± 1.9

Conclusions: We observed TEG® parameters variations whereas the TGT was constant throughout the study period. Our results suggest that TEG® changes mainly reflected the increase in fibrinogen level and not an increase in thrombin generation and therefore an hypercoagulable state in patients with sepsis or SIRS.

Vascular Biology

VAS01

Thrombus Formation and Platelet Dynamics Visualization by Multi-scale in vivo 1P, 2P Microscope, and On-chip Imager

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Background: Due to the inabilities of long-time and non-invasive *in vivo* monitoring, cellular mechanisms of cardiovascular events were still unclear.
Aims: We aimed to predict for cardiovascular events for daily monitoring and health-care by imaging approach.
Methods: We made minimum-invasive three devices to cover from micro to macro imaging for microcirculation in mice and humans.
Results: First one is high resolution imaging system based on non-linear optics. Real-time, multi-color XYZT multi-photon imaging enabled us to visualize single platelet behavior, morphological changes, and elucidate thrombus formation in cardiovascular events. Intracellular events including granule secretions, and organelle structures were also visualized. We also elucidated the platelet dynamics by pattern-matching based tracking software.
Second, macro imaging system for awake mice was developed, and free behavior monitoring revealed the tight association between metabolism and vascular reactions for daily stress.. Fluorescent imaging from body surface using 8K CMOS camera, image intensifier, and macro-lens enabled us to visualize cellular dynamics without anesthesia.
Third, wearable and implantable devices for long-time recording were developed using lens-less and on-chip technologies.
We utilized these system with light-manipulation technique, to induce thrombus or inflammation reactions.

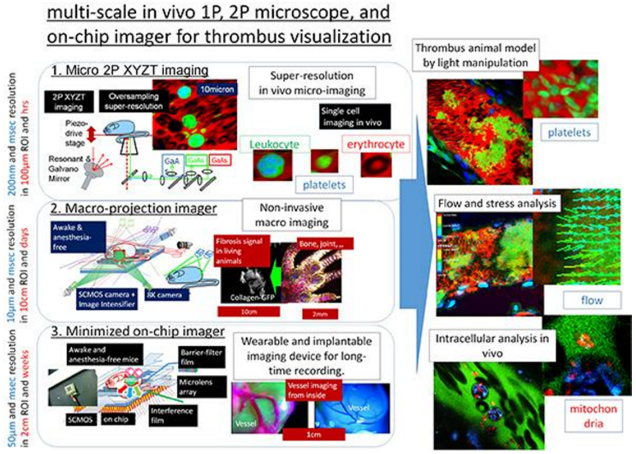

Figure 1

Conclusions: In sum, we developed multi-scale imaging system which can evaluate the therapeutic strategies against thrombotic and inflammatory processes in adult-common disease.

VAS02

Control of protein disulfide isomerase by nitric oxide

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Background: Protein disulfide isomerase (PDI) serves an essential role in thrombus formation and PDI inhibition is being evaluated in clinical trials as an anticoagulant. Yet little is known about endogenous regulation of PDI in the vasculature.
Aims: To determine whether PDI and other vascular thiol isomerases are controlled by nitric oxide (NO) through S-nitrosylation and assess whether thiol isomerases are effectors of NO in maintaining vascular quiescence.
Methods: The effect of nitrosylation of PDI on platelet and endothelial cell function as well as on thrombus formation *in vivo* was evaluated.
Results: Cysteines within the CGHC motif of PDI perform disulfide shuffling. Studies with PDI active site mutants showed that thiols within this motif bind NO, forming S-nitrosylated PDI (SNO-PDI). Functional studies showed that SNO-PDI has decreased oxidoreductase activity. S-nitrosylation of ERp5 and ERp57 also reduced their oxidoreductase function. Modulation of NO levels in endothelium using eNOS inhibitors and substrates showed that NO levels are inversely proportional to endothelial surface thiol isomerase activity. Incubation of endothelial cells with S-nitrosylated PDI facilitated transfer of NO to endothelial cell surface proteins such as αVβ3. Inhibition of PDI by small molecules or by exposure to NO donors blocked thrombin generation on endothelium. Evaluation of the effect of NO on platelets showed that platelet PDI can be S-nitrosylated. Addition of NO to resting platelets abolished reductase activity on the platelet surface. Purified SNO-PDI transferred NO to surface proteins such as αIIbβ3 and inhibited platelet aggregation and P-selectin expression. Infusion of SNO-PDI into mice blocked laser-induced thrombus formation as evaluated by intravital microscopy.
Conclusions: These studies demonstrate regulation of PDI function as a new mechanism by which NO maintains vascular quiescence.

VAS03

CD146 deficiency leads to accelerated atherosclerosis in mice through upregulation of RANTES

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Background: The progression of atherosclerosis is based on the continued recruitment of leukocytes in the vessel wall. The previously described role of CD146 in leukocyte infiltration *in vitro* suggests a role for this endothelial junction molecule in the pathogenesis of atherosclerosis. However, its involvement in atherosclerotic plaques formation has never been investigated.
Aims: We evaluated the role of CD146 in atherogenesis.
Methods: CD146 -/-/ApoE -/- and ApoE -/- mice were fed a Western diet for 24 weeks and were monitored for aortic wall thickness using high frequency ultrasound.
Results: The arterial wall thickness was significantly higher in CD146 deficient mice. We evidenced a significant increase of atheroma in both total aortic lesion and aortic sinus of CD146 deficient mice. In addition, atherosclerotic lesions were more inflammatory since CD146 deficient plaques contained more neutrophils and more macrophages. During atherosclerosis, circulating neutrophils were significantly increased in the absence of CD146. Consistent with the higher recruitment of inflammatory cells to the atheroma, we demonstrated that RANTES was up-regulated in CD146 deficient atherosclerotic arteries. In addition, CD146 deficient mice presented significant higher levels of circulating RANTES during atherosclerosis. Finally, we showed that macrophages were the source of RANTES and its production by CD146 null macrophages was also significantly increased through a mechanism dependent of p38-MAPK signaling pathway.
Conclusions: Our data indicate that CD146 deficiency is associated with the upregulation of RANTES and increased inflammation of atheroma, which could influence the atherosclerotic plaque fate. Thus, these data identify CD146 as a potential new target for atherosclerosis treatment.

VAS04

SGK1 deficiency protects from neointima formation via reduction of Ca^{2+}-dependent VSMC migration

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Background: Vascular inflammation and neointima formation require complex cellular signaling in inflammatory cells and especially vascular smooth muscle cells (VSMC) infiltrating the arterial vessel wall after vascular injury.
Aims: Aim of the present study was to determine whether Serum- & glucocorticoid-inducible kinase 1 (SGK1) is causally involved in development and progression of neointima formation as well as to identify underlying cellular mechanisms.
Methods: *In vivo* vascular injury; immunohistochemistry; confocal microscopy; immunoblotting; Boyden chamber; RT-PCR.

Results: Neointima formation in carotid arteries 4 weeks after vascular injury is significantly reduced in ApoE/SGK1 double knockout mice ($apoe^{-/-}sgk1^{-/-}$) compared to ApoE deficient mice ($apoe^{-/-}sgk1^{+/+}$) after treatment with cholesterol rich diet for 8 weeks. Vascular inflammation represented by infiltrating $CD45^+$ leukocytes, $CD3^+$ lymphocytes and Mac-3^+ macrophages was significantly diminished in $apoe^{-/-}sgk1^{-/-}$ mice compared to $apoe^{-/-}sgk1^{+/+}$ mice. Impaired neointima formation was paralleled by a significantly abrogated infiltration with $aSMA^+$ positive VSMC in $apoe^{-/-}sgk1^{-/-}$ mice. Expression of Ca^{2+} channel Orai1 in VSMC of $sgk1^{-/-}$ mice was significantly abolished. The defective Orai1 expression in $sgk1^{-/-}$ VSMC was paralleled by an abrogated migration of $sgk1^{-/-}$ VSMC *in vitro*. Orai1 mRNA level were significantly reduced in $sgk1^{-/-}$ VSMC pointing to a transcriptional regulation of Orai1 by SGK1 in VSMC. Nuclear translocation of transcription factor NF-κB subunit p50 was significantly diminished in $sgk1^{-/-}$ VSMC suggesting that VSMC Orai1 expression, critically important to migratory properties of VSMC, is under transcriptional control of SGK1 via NF-κB.
Conclusions: SGK1 plays a pivotal role in vascular inflammation and neointima formation after arterial vascular injury. SGK1 influences migration of VSMC by transcriptional regulation of Ca^{2+} channel Orai1 expression via transcription factor NF-κB.

VAS05

Lysine-rich histones induce weibel-palade body exocytosis through a calcium, caspase and charge-dependent mechanism

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Background: Histones are proinflammatory and procoagulant proteins released during cell necrosis/apoptosis and during NET formation. Histones induce platelet activation through Toll-like receptors 2 and 4 (TLR-2/4) and are cytotoxic to endothelial cells (ECs) at high concentrations. We previously demonstrated that histones induce Weibel-Palade body (WPB) exocytosis *in vitro* and *in vivo*. The mechanisms underlying histone interactions with ECs and cellular signalling that mediate WPB release are unknown. We hypothesize that histones may interact with ECs in a charge-dependent manner and that WPB exocytosis is associated with calcium mobilization in response to histone-mediated cytotoxicity.
Aims: To determine the extracellular and intracellular mechanisms by which histones induce WPB exocytosis.
Methods: Human umbilical cord vein ECs (HUVECs) and blood outgrowth ECs (BOECs) were pre-treated with selective antagonists or inhibitors for 1 h and then stimulated with PMA (100 nM), lysine-rich histones (HK, 12.5 μg/mL) or staurosporine (200 nM) for 2 h. The WPB constituent angiopoeitin-2 (ANG-2) was quantified from cell-supernatants by ELISA.
Results: Treatment of ECs with HK increased propidium iodide uptake (22.5% increase). Inhibition of apoptosis using a pan-caspase inhibitor (Z-VAD-FMK) inhibited HK (89-101%, $P = 0.02$) and staurosporine (85-92%, $P = 0.03$) induced ANG-2 release from ECs. Chelating calcium using BAPTA-AM also inhibited ANG-2 release in response to HK (89-92%, $P = 0.01$) and PMA (67-76%, $P = 0.008$). TLR-2/4 could not be identified on the surface of ECs and blocking TLR-2/4 did not protect against the release of WPBs. Pre-incubating ECs with the polycationic protein protamine sulfate (88-98%, $P = 0.003$) or annexin V (to block exposed phosphatidylserine residues) inhibited the release of ANG-2 by HK (107%, $P = 0.006$).
Conclusions: These studies demonstrate that histones cause WPB exocytosis in a calcium, caspase and charge-dependent manner. This axis may be a target in prevention of inflammation-induced thrombosis.

VAS06

Significant miRNA alterations in human endothelial cells as a result of *Staphylococcus aureus* infection

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Background: Sepsis is the leading cause of death in ICUs and is commonly caused by the opportunistic bacteria *Staphylococcus aureus*. In the bloodstream *S. aureus* binds to the inner lining of blood vessels, the endothelium, triggering endothelial dysfunction and inducing a major host inflammatory response. Despite high mortality rates, little is known about the signals leading to this reaction. miRNA are small non-coding RNA predicted to regulate 60% of the human genome including that of the endothelium.
Aims: The aim of this research is to ascertain if an alternative endothelial miRNA profile is produced by *S. aureus* infection and to investigate resulting regulation that may cause endothelial dysfunction.
Methods: Endothelial cells were sheared at 10 dynes/cm^2 and treated with plasma and TNFα to mimic conditions of sepsis. Total RNA was extracted from uninfected and infected cells using the mirVANA isolation kit. Using human specific miRNA primers, pre-amplified RT-qPCR was used to determine each profile and miRNA with significant expression changes were verified ($RQ = 2\text{-}\Delta\Delta Ct$). Potential mRNA targets were established using online databases, with targets considered if 3 confirmed the prediction. A proliferation assay compared cell numbers of uninfected and infected cells following a 24 h infection.
Results: 93 miRNA were differentially expressed, of which 35 were up- and 58 were down-regulated ($N = 4$, $P < 0.05$). KEGG pathway analysis demonstrates significant enrichment of targets in Linoleic Acid Metabolism (8 targets, $P = 0.0007$) and the MARK Signalling Pathway (3 targets, $P = 0.006$), pathways which may act in the dysregulation of inflammation and multiple organ failure associated with sepsis. Additional targets of interest were linked to vascular permeability and proliferation both of which are worsened by infection.
Conclusions: This atypical miRNA profile may explain how *S. aureus* causes endothelial dysfunction with abnormal intrinsic signalling leading to improper regulation of proliferation and permeability.

VAS07

Gain-of-function mutation in TRPV4 identified in patients with osteonecrosis of the femoral head

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Background: Osteonecrosis of the femoral head is a debilitating disease that involves impaired blood supply leading to femoral head collapse and end-stage osteoarthritis. The disease is poorly characterized with

mutations in only one gene previously identified in inherited osteonecrosis.

Aims: Treatment options for osteonecrosis are limited, highlighting the need for an improved understanding of disease pathogenesis and identification of new therapeutic targets.

Methods: We report a Canadian family of Greek origin with four siblings affected with osteonecrosis of the femoral head. Candidate genes were identified by whole exome sequencing followed by variant filtering. Sanger sequencing was carried out using genomic DNA. Fibroblasts from the proband and HEK293 cells were used for Western blotting. mRNA expression was determined by qPCR. Ca^{2+} signals were imaged by fluorescence microscopy.

Results: We present a novel c.2480_2483delCCCG frameshift deletion followed by a c.2486T>A substitution in one allele of the transient receptor potential vanilloid 4 (TRPV4) gene. *TRPV4* encodes a Ca^{2+} permeable cation channel known to play a role in vasoregulation and osteoclast differentiation. While pathogenic *TRPV4* mutations affect the skeletal or nervous systems, association with osteonecrosis is novel. Functional measurements of Ca^{2+} influx through mutant TRPV4 channels in HEK293 cells and proband fibroblasts identified a TRPV4 gain-of-function caused by longer channel openings. Ca^{2+} overload can lead to endothelial dysfunction and vasoconstriction, resulting in bone loss. TRPV4-mediated vasoconstriction was recently demonstrated in the vascular endothelium by G Protein-Coupled Receptor potentiation of TRPV4 through COX-dependent prostanoid production and thromboxane receptor activation.

Conclusions: These findings identify a novel *TRPV4* mutation implicating TRPV4 and altered calcium homeostasis in the pathogenesis of osteonecrosis while reinforcing the importance of TRPV4 in bone diseases and vascular endothelium.

VAS08

Endothelial cells are important players in the pathogenesis of thrombosis in myeloproliferative neoplasms

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Background: BCR-ABL-negative Myeloproliferative Neoplasms (MPN) are acquired haematological diseases. They result from the transformation of a hematopoietic stem cell with hyperproliferative differentiation due to a $JAK2^{V617F}$ point mutation in most cases. This mutation results in activation of the cytokine receptor/JAK2 pathway. Although arterial and venous thrombotic incidents are the most common causes of morbidity and mortality in patients with MPNs, the events causing these clotting abnormalities remain unclear. Recent studies have demonstrated the presence of $JAK2^{V617F}$ not only in blood cells but also in endothelial cells (EC) in some patients with thrombotic complications.

Aims: To assess whether $JAK2^{V617F}$ ECs promote a prothrombotic phenotype and participate to thrombus formation.

Methods: We studied ECs $JAK2^{V617F}$ involvement in leucocyte adhesion in static and dynamic conditions. We used either cells isolated from the vein of the umbilical cord (*HUVEC*) or cells isolated from the coronary arteries (*HCAEC*). Both were stably transfected with the human $JAK2^{V617F}$ and GFP. Cells transfected with $JAK2^{WT}$ and GPF served as control.

Results: We showed that

1) mononuclear cells (MNC) and polymorphonuclear neutrophils are significantly more adhesive on monolayer HUVEC $JAK2^{V617F}$ (for both cell types $P < 0{,}05$) and HCAEC $JAK2^{V617F}$ ($P < 0{,}01$ and $P < 0{,}001$) in static condition;

2) MNC are more adhesive on HUVEC $JAK2^{V617F}$ preteated with TNFα (1 ng/mL) overnight ($P < 0.01$) in dynamic conditions;

3) Immunofluorescence staining revealed increased P-selectin expression at the surface of $JAK2^{V617F}$ HUVEC;

4) Pretreating HUVEC and HCAEC $JAK2^{V617F}$ with anti-P-Selectin blocking mAb reduces MNC and PMN adhesion.

Conclusions: This study demonstrates that $JAK2^{V617F}$ ECs have a proadhesive phenotype both in static and dynamic condition due to P-Selectin overexpression. This could contribute to thrombotic events and *in vivo* experiments are currently ongoing.

VAS09

Soluble CD146 priming boosts survival and regenerative properties of endothelial colony-forming cells

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Background: Endothelial colony-forming cells (ECFC) constitute an endothelial progenitor fraction with a promising interest for the treatment of ischemic cardiovascular diseases.

Aims: As soluble CD146 (sCD146) was described as a new factor promoting angiogenesis, we examined whether sCD146 priming could improve the therapeutic potential of ECFC and defined the involved mechanism.

Methods: The effect of sCD146 priming on ECFC properties was investigated *in vitro* and *in vivo*. To this end, proliferation/migration experiments, immunoprecipitation experiments and DNA fragmentation assays were performed. In addition, a mouse model of matrigel plug, that mimicked a hypoxic environment, and a mouse model of hind limb ischemia were used.

Results: Soluble CD146 priming improves the survival of ECFC and boosts their revascularization potential. The observed effects are mediated through a signalosome, located in lipid rafts, containing specifically the short isoform of CD146 (shCD146), angiomotin, and VEGFR1/VEGFR2 and involves the transcription of genes related to angiogenesis (eNOS) and cell viability (FADD, Bcl-xl).

Conclusions: These findings establish that activation of shCD146, in particular with sCD146 priming, constitutes a new pathway to improve ECFC regenerative properties for the treatment of cardiovascular diseases.

VAS10

Activated protein C (APC) down regulates neutrophil extracellular traps (NETs): potential contributing mechanism for anti-inflammatory and antithrombotic effects Of APC

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Background: At sites of microbial infections, activated neutrophils can expel their nuclear content to form microbicidal protein-exposing neutrophil extracellular traps (NETs). This process of NETosis provides a

structural framework for pathogen clearance. Studies suggest that NETs also promote thrombus formation. The exact mechanism of NETosis, including its regulation, if any, is unclear. Activated protein C (APC) is a natural antithrombotic, anti-inflammatory and cytoprotective blood enzyme that inhibits the migration of neutrophils, but its effect on NETosis is unclear.

Aims: To determine the effect of exogenous APC on phorbol myristate acetate (PMA)-induced NETs formation.

Methods: Human neutrophils were purified from peripheral blood and allowed to adhere to a fibronectin-coated surface. Neutrophils were then pretreated with increasing concentrations of human APC prior to stimulation with PMA to induce NETosis. NETosis was quantified using the DNA-binding dye, Hoescht 33342, as well as neutrophil elastase and citrullinated histone. The area of DNA was quantified via fluorescence microscopy and MATLAB-based image analysis.

Results: APC prevented NETosis induced by the protein kinase C activator, PMA. The ability of APC to prevent NETosis was reversed after pretreatment with the protease inhibitor, PPACK. Function blocking antibodies to EPCR, PAR-3 and Mac-1 and pharmacological inhibitors of the PDK and PLC signaling pathways prevented APC from inhibiting PMA-induced NETosis. Recombinant APC with several mutations in and near the protease domain failed to prevent the ability of PMA to induce NETosis.

Conclusions: Our data suggest that *ex vivo*, exogenous APC can down-regulate PMA-induced NETosis, and that inhibition of NETosis by APC involves in part, EPCR and/or Mac-1, cleavage of PAR-3, and by signaling via the GPCR/PDK/PLC cascade. Our data help to identify an additional possible link to the anti-inflammatory and antithrombotic activities of APC in immunothrombosis.

VAS11

Platelet-derived Factor V Is an important determinant of ischemia-mediated angiogenesis in a mouse hindlimb model

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Background: Coagulation factor V (fV) is distributed in plasma and platelet pools distinguished by physical and functional differences. fV has been extensively studied for its roles in coagulation. The roles of fV in other physiological pathways remain understudied.

Aims: There is no report on the roles of FV in angiogenesis. In the current study, we demonstrate that platelet FV could play an important role in angiogenesis.

Methods: In the current study, we report that platelet fV is critical for the regulation of angiogenesis in a mouse model of hind-limb ischemia. Hindlimb ischemia was produced in mice by femoral artery ligation in transgenic mice, with different level of fV gene expression restricted to either the plasma or platelets.

Results: The hindlimb blood flow perfusion in mice with higher platelet fV was significantly greater. The expression of major angiogenesis-related factors were correlated with the level of fV in ischemia. Furthermore, using a platelet depletion/transfusion procedure, transfusion of platelets with higher level of fV into transgenic mice with undetectable platelet fV significantly rescued the ischemia-mediated impairments in blood flow perfusion. Immunohistochemical analysis also indicated markedly increased capillary formation in ischemic muscle of mice with higher platelet fV. Moreover, thrombin activity was significantly higher in the mice with higher platelet fV. Platelet with higher level of fV demonstrated significantly higher pro-migratory activity in an endothelial cells migration assay. The hindlimb blood flow perfusion was significantly blocked by thrombin inhibitor.

Conclusions: These findings suggest that platelet-derived fV contributes to the control of angiogenesis, and is likely associated with thrombin generation due to platelet-derived fV.

VAS12

What is the best animal model to specifically target endothelial cells, without concomitant involvement of the hematopoietic lineage?

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Background: With the use of the Cre/Lox system, various endothelial specific transgenic mouse lines have been generated. Given that most endothelial promoters are also expressed in hematopoietic stem cells in the embryo, inducible Cre-recombinase models offer the advantage to avoid hematopoietic penetrance. Inducible *Pdgfb-iCreERT2* and *Cdh5 (PAC)-CreERT2* transgenic mice are widely used for endothelial targeting. However issues remain in term of recombination efficiency and specificity regarding hematopoietic cells.

Aims: To determine which mouse model to choose when a strong expression of a transgene is required in adult endothelial cells from various organs, without concomitant expression in hematopoietic cells.

Methods: We used two complementary approaches: the use of *mT/mG* mice to report for homogeneity and flexed *JAK2 ($JAK2^{V617F/WT}$)* mice to report for specificity regarding hematopoietic cells, as expression of JAK2V617F in hematopoietic stem cells gives rise to a myeloproliferative disease and could reveal a small subset of recombined hematopoietic stem cells.

Results: We showed that adult *Cdh5(PAC)-CreERT2* mice can be used with however the precaution that recombination is highly variable among mice. We found that *Pdgfb-iCreERT2* mice are appropriate for most endothelial research fields except liver studies, as hepatic sinusoid endothelial cells are not recombined. Surprisingly we observed, 2 months after induction of Cre-mediated recombination, that all *Pdgfb-iCreERT2;$JAK2^{V617F/WT}$* mice developed a myeloproliferative neoplasm that was related to the presence of JAK2V617F in hematopoietic cells. We suggest that *Pdgfb-iCreERT2* transgenic mice should be used within the first month after induction of Cre-mediated recombination, so that penetrance of recombined hematopoietic cells does not occur.

Conclusions: This study highlights major differences in term of recombination efficiency and specificity between 2 endothelial inducible transgenic lines.

VAS13

Gaining insight into the early signals generated in human endothelial cells as a result of bacterial infection

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Background: Sepsis is a major challenge in the intensive care unit, where it is one of the leading causes of death. The endothelium is a

major target of sepsis-induced events and vascular endothelial damage accounts for much of the pathology of septic shock. *Staphylococcus aureus* and *Escherichia coli* are the most common bacteria isolated from sepsis patients. The inflammatory response plays a key role in the sepsis phenotype and an excessive or sustained inflammatory response contributes to the tissue damage and death.

Aims: The aim of this work was to identify key cytokines, chemokines and acute phase proteins released from Human Aortic Endothelial Cells (HAoEC's) common to both *S. aureus* and *E. coli* infection.

Methods: HAoEC's were sheared at 10 dynes to represent a physiological model. The sheared HAoEC's were infected for 24 hr with *E. coli* and *S. aureus*. Using a multiplex cytokine array we determined a profile of secreted cytokines, chemokines and acute phase proteins released following infection.

Results: CXCL1, GM-CSF and G-CSF are essential for angiogenesis, migration and proliferation in endothelial cells and were significantly increased following S. aureus and E. coli infection of HAoEC's. The release of sICAM-1 was also significantly increased from HAoEC's in response to S. aureus and E. coli infection. Released sICAM-1 is capable of inhibiting lymphocyte attachment to HAoEC's. Acute phase Serpin E1 (plasminogen activator inhibitor - 1) is increased in both infected models. Serpin E1 is a serine protease inhibitor that inhibits plasminogen activators which are essential in the mechanical breakdown of thrombi which forms as a result of either S. aureus or E. coli binding to platelets.

Conclusions: Identification of the key signal mediators helps us understand the intraconnected molecular cross talk that occurs following bacterial binding to human endothelial cells. Understanding the signals that drive sepsis at an early stage is critical for the development of novel therapeutics to help treat sepsis.

VAS14

development of a platform to quantify the colocalization of neutrophil extracellular DNA and coagulation factors using immunofluorescence-based microscopy

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Background: Neutrophils, the most populous innate immune cell type, are the first responders to sites of infection and inflammation. Neutrophils can release their DNA to form extracellular traps (NETs), webs of DNA and granular proteases that contribute to pathogen clearance. At present, the study of NETs is limited to the qualitative analysis of fluorescence microscopy-based images.

Aims: Develop a quantitative method to measure the spatial distribution of DNA and colocalization of coagulation factor binding to NETs.

Methods: Human neutrophils were purified from peripheral blood, bound to fibronectin and treated with the PKC-activator phorbol myristate acetate (PMA) to induce NETs formation. Samples were incubated with purified coagulation factors or plasma before staining with a DNA-binding dye and coagulation factor-specific antibodies. The spatial distribution of DNA and coagulation factor proteins was imaged via fluorescence microscopy and quantified via a custom-built MATLAB-based image analysis algorithm. This algorithm first establishes global thresholding parameters on training set fluorescence image data to then systematically quantify intensity profiles across treatment conditions. Quantitative comparison of treatment conditions is enabled by the normalization of fluorescent intensities by the number of cells per image to determine the percent and area of DNA and coagulation factor binding per cell.

Results: Upon stimulation with PMA, NETs formation resulted in an increase in the area of DNA per cell. The coagulation factor fibrinogen bound to both the neutrophil cell body as well as NETs, while prothrombin, FXa and FVIIa binding was restricted to the neutrophil cell body. Activated protein C (APC), but not Gla-less APC, bound to NETs in a punctate manner. Neither FXIIa nor FXIa were found to bind NETs.

Conclusions: We have developed a quantitative measurement platform to define the spatial localization of fluorescently-labeled coagulation factor binding to neutrophils and extracellular DNA during NETosis.

VAS15

Platelet function and microparticles levels for patients with paroxysmal and persistent atrial fibrillation including during acute episodes

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Background: Arterial thromboembolism constitutes a major risk of atrial fibrillation (AF) requiring antithrombotic therapy. Platelets and microparticles (MPs) are important for hemostasis and thrombosis, their role during AF is not well known.

Aims: To characterize platelet aggregation and to evaluate MPs in the left atrium (LA) of AF patients including during acute episodes.

Methods: Paroxysmal (n = 21) and persistent (n = 17) AF patients referred for catheter ablation were recruited. Patients in sinus rhythm (n = 10) were induced in AF and citrated blood was taken from the LA before and after the acute episode (20 min). Platelet aggregation was performed in PRP using ADP (10 μM), TRAP6-mer (10 μM), collagen (2 μg/ml), and ristocetin (1.5 mg/ml). Levels of procoagulant (TF+) and fibrinolytic MPs were determined in plasma by functional assays.

Results: Persistent AF patients showed higher maximal aggregation for TRAP compared to paroxysmal patients (75.3 ± 12.3% vs 58.4 ± 16.0% $P = 0.005$). Aggregation was more reversible in paroxysmal than persistent AF (40.5 ± 18.8% vs 27.7 ± 21.4% $P = 0.016$). Procoagulant TF-dependent MP activities were reduced compared to controls, and slightly more so in persistent than in paroxysmal AF patients (8.0 ± 9.0 vs 13.2 ± 10.8 fM TF $P = 0.076$). Levels of fibrinolytic MPs were unchanged between paroxysmal, persistent AF patients and controls. During acute episodes of AF, aggregation with TRAP (73.6 ± 16.0% vs 66.8 ± 16.0%, $P = 0.009$) and collagen (74.2 ± 13.4% vs 66.7 ± 15.8% $P = 0.023$ was increased but the activity of procoagulant MPs decreased (15.9 ± 11.6 *vs* 5.3 ± 6.3 fM TF $P = 0.03$). Levels of fibrinolytic MPs remained stable.

Conclusions: The lower response of platelets to TRAP activation in paroxysmal AF patients can correspond to desensitization from a previous contact with thrombin but the process is reversed in acute episodes. Lower levels of procoagulant MPs for AF patients and their disappearance during acute AF measured in the LA are in favor of their consumption in hemostatic activation processes.

VAS17

Biological reference materials to standardize measurements of extracellular vesicles

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Background: Most current studies on extracellular vesicles (EVs) use synthetic reference materials (SRM) to increase the comparability of EV measurement results between instruments, institutes and over time. As the physical properties of SRM, such as silica and polystyrene beads, differ from EVs, the use of SRM as size references for EVs may lead to erroneous inclusion of EV subpopulations and/or non-EV particles.
Aims: Here, we present an overview of 1) the optimal physical properties of biological reference materials (BRM) as surveyed from people working with EVs, 2) choices for such BRM based on a literature search, and 3) characterization of the physical properties of a promising potential erythrocyte-derived BRM, nanoerythrosomes (NE).
Methods: To discover the user preferences of the physical properties of BRM, a questionnaire was sent to 46 laboratories working with EVs (44% reply rate).
Taking into consideration the desired properties for BRM and the feasibility for a large scale production, NE, particles created by (mechanical) disruption of erythrocytes, were chosen for further studies. Large scale production of NE was tested by three different methods.
Results: According to the survey response, the most used method to measure EVs is flow cytometry (90%), and biochemical resemblance to EV was considered to be the most important property of BRM.
The results of literature search of BRM could be divided to three categories: 1) naturally occurring BRM (e.g. isolated EV populations, marine bacteria), 2) BRM from purified biochemical components or membranes (e.g. liposomes and oil droplets), and 3) miscellaneous biological particles (e.g. lipoparticles).
Two important physical properties, i.e. the size distribution and refractive index, of the produced NE were comparable to EVs satisfying some of the key aspects of an optimal BRM.
Conclusions: Further investigation of the BRM choices, novel and those reported in the overview, will be needed to find BRM which match multiple standardization properties of the EVs.

VAS18

SIRT1 Deficiency in endothelial progenitor cells drives pro-senescent microparticles release through MKK6 upregulation

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Background: Preterm birth is recognized as an independent risk factor for cardiovascular disease occurring later in life. We previously reported that endothelial colony forming cells isolated from preterm neonates (PT-ECFC) display SIRT1-driven accelerated senescence, a mechanism that may contribute to impaired endothelial repair. Cellular senescence alters the secretory profile including soluble factors and microvesicles. However, whether dysregulated microparticles biogenesis participates in endothelial senescence, remains to be investigated.
Aims: We investigate whether the accelerated senescence of PT-ECFC could lead to a senescence associated accelerated phenotype (SASP) involving the release of pro-senescent microparticles (MP).
Methods: MP were analyzed in conditioned media from PT (n = 25) and term ECFCs (CT-ECFCs, n = 18) using qNano technology or flow cytometry, respectively. Signaling pathways involved in SIRT1-mediated MP release were highlighted across transcriptomic analysis and various strategies of SIRT1 expression modulation. Purified MP were tested for their capacity to induce senescence in first passage endothelial cells using β-galactosidase staining, proliferation assay and Western blot analysis of senescence markers.
Results: Increased MP release was observed in conditioned media from PT-ECFC and prevented by resveratrol treatment or SIRT1 overexpression. We demonstrated that MP biogenesis is dependent on SIRT1 deficiency that activates $MKK6/p38^{MAPK}$ signaling pathways. MP produced by PT-ECFC take part of an inflammatory secretory phenotype, including IL6. Interestingly, they are predominantly involved in the induction of senescence markers into naive endothelial cells.
Conclusions: SIRT1 silencing in ECFC lead to a SASP that are associated to the biogenesis of pro-senescent MP. Deciphering the role of MP in SASP may improve our understanding of how endothelial senescence determines cardiovascular risk.

VAS19

Natural killer cells are potent effectors of thromboinflammatory endothelial dysfunction associated with antibody-mediated transplant vasculopathy

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Background: Antibody-mediated rejection is a major cause of allograft loss. Donor specific alloantibodies (DSA) are involved in the progression of kidney allograft vascular lesions and transplant-associated thrombotic microangiopathy. Understanding the mechanisms that determine DSA pathogenicity in a given patient is essential to identify patients at risk to develop graft failure.
Aims: We aimed to investigate whether antibody-driven Natural Killer cell cytotoxic activity (NK-ADCC) can be identified as a mechanism promoting endothelial dysfunction in kidney transplant recipients (KTR).
Methods: A flow cytometry Cellular Humoral Activation Test (NK-CHAT) was designed to evaluate recipient- and Antibody-dependent NK-cell activation towards cells expressing target antigens.
Results: Evaluation of NK-ADCC towards Rituximab-coated cells revealed high inter-individual variability of humoral immune responses in KTR, suggesting that differential NK-ADCC potential may be associated to efficiency of Rituximab desensitization therapies

or intensity of DSA- mediated allograft vasculopathy. The level of CD16/FcRIII engagement was identified as a specific index of DSA-mediated NK-ADCC towards endothelial cells expressing target HLA alloantigens. NK-cell recognition of DSA-coated target cells was associated with enhanced cytolysis of allogeneic glomerular cells. FcR-driven NK-cell ADCC recognition of antibody coated-endothelial or tumor cell targets significantly induced Tissue Factor (TF) transcript levels and release of microparticles with TF-dependent pro-coagulant activity *in vitro*.

Conclusions: Our data suggest that upon graft vascular exposure to DSA, NK-ADCC may promote pro-thrombotic and pro-inflammatory conditions associated with graft vascular injury. Through indexing of complement independent mechanisms that control intensity of IgG-dependent immune target cell recognition, non-invasive NK-CHAT may represent a valuable companion assay allowing better appraisal of humoral and thrombotic risk on an individual basis.

VAS20

Microparticles from acute promyelocytic leukemia generate plasmin in a urokinase-dependent manner

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Background: Acute promyelocytic leukemia (APL) is associated with a high rate of coagulopathy leading to hemorrhagic events. Microparticles (MPs) have been identified as a new actor in the hemostasis equilibrium. Besides their procoagulant activity, a capacity to generation plasmin has been recently described on MP from endothelial, leucocyte and tumoral origins.

Aims: Thus, we hypothetized that APL-derived MPs may vectorize both procoagulant and fibrinolytic molecules and contribute to the hemostasis disorders encountered in the APL patients.

Methods: Using APL cell line MPs (NB4), we characterized for the first time the plasminogenolytic system present on these MPs. Altogether results from flow cytometry, ELISA and fibrin zymography demonstrated the presence of urokinase (uPA) and its receptor (uPAR).

Results: UPA was present both in free form or in complex with the plasminogen activator inhibitor (PAI-1). In contrast, no tissue type plasminogen activator (t-PA) was detected. Using the uPA/uPAR system, APL-MPs actively generate plasmin as shown by zymography, a plasmin specific chromogenic test and by specific inhibitions with a2-antiplasmin, anti-uPA antibodies or removal of uPAR from the MP surface. These results were confirmed on MPs purified from APL patients. On a pilot cohort of 12 APL patients, the plasmin generation capacity of myeloïd MPs (MP-PGC) measured by a new assay immunomagnetic separation and chromogenic detection of plasmin was significantly increased compared to healthy donors (20.4 [2 -87] pM vs 8.5 [0-13] pM, $P = 0.03$, respectively). Interestingly, MP-PGC was positively correlated to a hemorrhagic score based on bleeding duration and was not correlated to the blast counts. This result need to be confirmed in a larger cohort currently being collected.

Conclusions: This work demonstrated that APL MPs convey plasminogen activators that may play a role in the hemostasis imbalance frequently observed in APL patients.

VAS21

Circulating lipoprotein (a) increases aortic-valve calcification

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Background: Lipoprotein (a) [Lp (a)] is involved in the initiation, progression and rupture of the atherosclerotic plaque. It impaired fibrinolysis due to its structural similarity to plasminogen and to promote monocytic activation. Aortic-valve calcification is related to one SNP in the lipoprotein(a) (LPA) locus (rs10455872). Elevated levels of circulating Lp(a) and valvular calcification are risk factors for the development of atherothrombotic disease and aortic-valve stenosis.

Aims: To identify a relationship between elevated Lp(a) and aortic-valve calcification, in patients attending the National Institute of Cardiology "Ignacio Chavez".

Methods: Thirty three consecutive patients who undergo an aortic-valve replacement were included in a cross-sectional study. Aortic-valve calcification was analyzed by scanning electron microscopy. The images obtained were analyzed with the software ImageJ1.5b. The plasma concentration of Lp(a) was performed by an ELISA kit following the manufacturer instructions. Statistical analysis was made using SPSS version 21 software. The study was approved by the institutional ethical and scientific committee.

Results: Lp (a) concentration had a median of 1.01 mg/mL (0.27-2.47) minimum and maximum respectively. Aortic-valve calcium had a median of 48.27 % (0.46-80.26) minimum and maximum respectively. Both variables had a Spearman correlation of 0.512 with a statistical significance of
$P = 0.02$.

Conclusions: The results allow us to identify a correlation between an increased plasma level of Lp (a) and an increased calcium deposit on the aortic-valves which suggest it could be translated into an increased risk factor for the development of aortic-valve stenosis.

VAS22

Association of nitric oxide synthase3 (NOS3) gene polymorphisms with acute myocardial infarction in Indian patients

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Background: Acute Myocardial infarction (AMI) is a major cause of death and disability worldwide. Several polymorphism of NOS3 has been reported and their association with AMI and other heart disease are conflict. Several studies have documented the role of NOS3 Polymorphisms in the pathogenesis of AMI. The genetic variation in genes coding for NOS3 increases the risk of AMI. The decrease levels of nitric oxide has been reported to be associated with NOS3 gene Polymorphism in Greece population AMI patients.

Aims: The aim of the study was to determine the association of polymorphism (Glu298Asp and T786C) with the plasma nitric oxide level in AMI patients as well as healthy control in India.
Methods: Total 100 consecutive patients with AMI and 100 age and sex matched healthy controls were the study subjects. Plasma levels of nitric oxide were identified by ELISA and the Polymorphisms of NOS3 gene was detected by PCR-RFLP.
Results: Plasma levels of nitric oxide were found to be significantly higher in healthy control (43.80 ± 6.28sd) than AMI patients (37.05 ± 6.75sd), ($P < 0.001$). The frequency distribution for Glu298Asp polymorphism among the AMI patients and healthy control was as follows: Glu/Glu, Glu/Asp, Asp/Asp genotypes were 60%,33%, 7%, and 73%, 23%, 4%, respectively ($P = 0.144$), For T786C polymorphism, T/T, T/C, C/C genotype were 60%, 36%, 4% and 67%, 32%, 1%, respectively
($P = 0.312$).
Conclusions: The decreased level of nitric did not show any association with the polymorphisms of NOS3 gene. A study with larger sample size is required to find out the association of phenotype with the genotype.

VAS23

Endothelial cells response to staphylococcus aureus: critical role of clumping Factor A in sepsis

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Background: The barrier function and antithrombotic properties of endothelial cells (ECs) are challenged by bloodstream infections. These phenomena are exacerbated in sepsis, often caused by gram positive bacteria (*S. aureus*), when interstitial oedema and altered coagulation may result in organ failure induced death. To date, the molecular interactions and associated intracellular signalling by which *S. aureus* induces endothelial cell dysregulation are not completely understood.
Aims: The aim of this study was to investigate the molecular interaction and intracellular signals generated following *S. aureus* binding to ECs.
Methods: Human aortic ECs were sheared at 10 dynes/cm^2 in the presence of plasma proteins and TNFα and exposed to bacteria. Intracellular Ca^{2+} was measured by epifluorescence (Fluo-4), and extracellular vWf was determined by immunofluorescence. Anaesthetized C57Bl/6 mice were injected with fluorescently labelled *S. aureus* and adhered bacteria were visualised within the mesenteric circulation *in vivo* using an inverted microscope.
Results: A clumping factor A-deficient *S. aureus* strain (ΔClfA) displayed reduced *in vitro* adhesion to ECs (-65 ± 6% vs. *S. aureus* WT, $P < 0.01$). Remarkably, *S. aureus* ΔClfA also exhibited significantly reduced binding to the endothelium *in vivo* using an animal model of sepsis (-84 ± 8% vs. *S. aureus* WT, $P < 0.01$). *In vitro* experiments showed that ClfA is capable of inducing a transient rise of intracellular Ca^{2+} (increased by 25 ± 2% vs. uninfected, $P < 0.05$) upon binding the ECs. Mobilisation of Ca^{2+} was rapidly accompanied by secretion of the Weibel Palade bodies in the ECs leading to exposure of vWf on the surface of the ECs (70 ± 20% increase vs. uninfected, $P < 0.05$).
Conclusions: We conclude that EC-ClfA interaction plays a critical role in *S. aureus* infection. Once bound signal generation results in exposure of vWf on the surface of ECs, thus amplifying the infective process. A better understanding of the molecular mechanisms will lead to novel therapeutics to treat sepsis.

VAS24

Acidic preconditioning improves endothelial progenitor cell survival and functionality under inflammatory conditions

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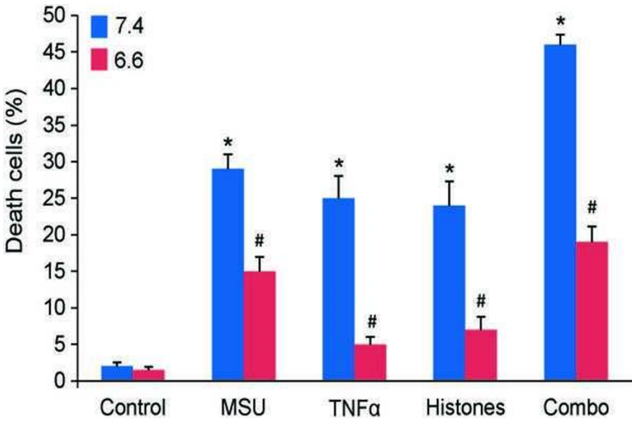
Background: We have previously demonstrated that acidic preconditioning exacerbates angiogenic responses of late outgrowth endothelial progenitor cells (ECFC).
Aims: In the present work, we aimed to analyze whether this strategy is also effective to improve ECFC functionality under stressful conditions typically observed in inflammation or cardiovascular diseases such as high glucose, monosodium urate (MSU) crystals, TNFa or extracellular histones.
Methods: Acidic preconditioning was achieved by incubating ECFC in EGM2, pH 6.6 for 6 h (preconditioned) and then medium was replaced by fresh EGM2, pH 7.4. ECFC incubation at pH 7.4 for 6 h was conducted as control. Viability (nuclear morphology analysis by fluorescence microscopy), wound healing (scratch assay) and tubule formation (matrigel) were analyzed 24 h after treatment. Data are shown as % of control, n = 3-4, * $P < 0.05$ vs. untreated control, # $P < 0,05$ vs. same condition at pH 7.4, one-way ANOVA.
Results: We found that while MSU crystals, TNFa, histones or the combination of these substances (combo) induced ECFC apoptosis and necrosis in a concentration-dependent manner at pH 7.4, high glucose medium (25 mM) failed to affect cell survival. Interestingly, the cytotoxic effect of MSU crystals (150 mg/ml), TNFa (100 ng/ml), histones (3 μM) or combo was significantly lower in preconditioned ECFC (Figure). Moreover, when a non-toxic concentration was used, high glucose (25 mM), MSU crystals (75 mg/ml), TNFa (20 ng/ml) or histones (1 μM) inhibited wound healing (70 ± 2*, 90 ± 1*, 64 ± 1* or 31 ± 2* %, respectively) and tubule formation (80 ± 1*, 69 ± 6*, 74 ± 2* or 69 ± 4* %), whereas no significant effect was observed in preconditioned ECFC.



Figure

Conclusions: In conclusion, preconditioned ECFC showed improved survival and angiogenic responses in the presence of harmful and inflammatory agents, suggesting that acidic preconditioning could be considered as an effective strategy to improve tissue regeneration in inflammatory and cardiovascular diseases where this process is highly impaired.

VAS25

Recanalization rates in patients with proximal deep venous thrombosis patients - a prospective observational study from a tertiary care hospital in North India

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Background: Outcome of DVT depends on early detection, institution of therapy as well as restoration of circulation.
Aims: Factors affecting the recanalization in patients with proximal DVT.
Methods: 25 adult patients with symptomatic first episode of DVT of the proximal veins of lower limbs (femoral and popliteal veins) were enrolled.DVT was diagnosed by duplex ultrasonography. which was also done at each follow-up visit at one and 3 months.Anticoagulation with heparin (UFH /LMWH) along with warfarin was initiated from the first treatment day. Heparin was stopped when an INR of >2 was maintained for at least 48 h and thereafter warfarin was continued for at least 6 months. Serial monitoring of INR was done to maintain an INR value of about 2.5 (desirable range, 2.0-3.0).
Results: The mean age of the enrolled patients was 39.9 ± 12.02 years with 55 % being males. Out of the 40limbs/20 patients (both diseased and normal limbs), 20 limbs (50%) which had complete occlusion at baseline, 8(40%) remained in the state of complete occlusion, 1(5%) completely recanalised, 11(55%) partial recanalised after 1 month of anticoagulation. Though not statistically significant, better recanalisation rates after 1 month of anticoagulation were observed in males, young patients (75% vs. 44.4%), BMI more than 25 kg/m2(55.5% vs. 50%), early anticoagulation initiation early (61.5% vs. 42.9%) and who attained early desired therapeutic INR (57.1% vs. 50%) At 3 months out of 34limbs/17 patients (both diseased and normal limbs), 18 limbs, which had complete occlusion in the baseline, 3(16.6%) remained in the state of complete occlusion, 3(16.6%) completely recanalised, 12(66.8%) partial recanalised. Again though not statistically significant, better recanalisation rates after 3 months of anticoagulation were observed in same patient cohort as above i.e. at 1 month of anticoagulation.
Conclusions: The factors that influence recanalization are poorly understood. Further studies are needed to study these factors in detail.

VAS26

Application of a clot based assay to measure the procoagulant activity of stored allogeneic red blood cell concentrates

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Background: Thrombotic effects are possible complications of red blood cell (RBC) transfusion. The generation and accumulation of procoagulant RBC extracellular vesicles (EVs) during storage may play an important role.
Aims: The objective of this study was to assess the interest of a simple phospholipid-dependent coagulation assay (STA®-Procoag-PPL) to evaluate the procoagulant activity of stored RBC and its evolution during storage.
Methods: This study was approved by our local ethics committee. After informed and written consent, 12 volunteer adult donors each gave 450 ± 50 mL of whole blood. EVs from these 12 RBC concentrates at 13 time-points of storage were isolated and characterized by quantitative and functional Methods
the hemolysis rate (direct spectrophotometry), the quantification and determination of cellular origin (flow cytometry) and the pro-coagulant activity (thrombin generation and STA®-Procoag-PPL assays) were assessed.
Results: An exponential increase of the number of RBC EVs was observed (mean at the day of collection: 1,779 EVs/μL, mean at the end of storage period: 218,451 EVs/μL). 97.6% were annexin V negative. Isolated EVs were able to initiate thrombin generation. They have a phospholipid-dependent procoagulant activity as measured by the STA®-Procoag-PPL assay. Results of the peak of thrombin and the STA®-Procoag-PPL were well correlated (partial r = -0.41. $p < 0.001$).
Conclusions: The STA®-Procoag-PPL is a potentially useful technique to assess the procoagulant activity of a RBC concentrate.

von Willebrand Factor

VWF01

Blood outgrowth endothelial cells from type 3 von willebrand disease patients display abnormal angiogenesis

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Background: Bleeding associated with angiodysplasia is a recognized complication in von Willebrand disease (VWD) patients and recently, von Willebrand factor (VWF) has been identified as a negative regulator of angiogenesis.
Aims: This study examines the effect of different *VWF* mutations in type 3 VWD on angiogenesis using patient-derived blood outgrowth endothelial cells (BOECs).
Methods: The study was approved by Queen's University REB and 10 healthy controls and 5 type 3 VWD patients gave consent. Bleeding scores, plasma VWF/FVIII levels and ABO were obtained and BOECs were isolated. BOECs were analyzed for VWF and angiopoietin-2 (Ang-2) secretion via ELISA and storage using confocal IF. Relative gene expression of VWF, Ang-2 and integrin β3 was determined using qRT-PCR. Angiogenic profiles of BOECs were assessed by adhesion to matrix proteins, proliferation, migration and tubule formation.
Results: Consistent with deficient plasma VWF levels (Table 1), VWD BOECs had little VWF secretion ($P < 0.0001$; Table 2) and diffuse VWF staining. Higher Ang-2 secretion in T3-1 and T3-2 ($P < 0.05$; Table 1) was associated with increased Ang-2 gene expression compared to controls (T3-1 = 227 and T3-2 = 513 fold-change). Ang-2 staining in type 3 BOECs was diffuse with little co-localization alongside VWF. Integrin β3 gene expression did not vary between control and VWD BOECs but, adhesion of VWD BOECs to the matrix proteins was slightly reduced ($0.51 < P < 0.91$), suggesting impaired integrin function due to β3 internalization by Ang-2. T3-3 was less proliferative after 6 days (4974 cells,
$P < 0.001$). T3-1, T3-2 and T3-5 were slower (0.33, 0.28 and 0.41 μm/min respectively; $P < 0.05$) but had better directionality (T3-1 = 0.64

Table 1 Patient characteristics. (Abstract VWF01)

Subject ID	Sex/Age (M/F year)	Blood Type	Bleeding Score	VWF:Ag (IU/mL)	VWF:RCo (IU/mL)	FVIII:C (IU/mL)	Nucleotide Change, HGVS	Amino Acid Change, HGVS
T3-1	F/21	O+	24	0.01	0.00	0.01	c.876delC, c.1255C>T	*p.Ser293 fs, Gln419**
T3-2	F/11	O+	13	0.01	0.03	0.01	c.1897T>C	*p.Cys633Arg*
T3-3	M/16	A+	24	0.01	0.03	0.01	c.1657dupT, c.8419_842dupTCCC	*p.Trp522 fs, Ser2807 fs*
T3-4	M/27	A+	29	0.02	0.01	0.01	c.3939G>A, c.5842 + 1 G>C	*p.Trp1313**, -
T3-5	F/19	O+	12	0.01	0.08	0.04	c.1729 + 3A>C	-

and T3-2 = 0.65, $P = 0.0001$). T3-1 and T3-2 had reduced tubule formation in Matrigel after 8 h (11.29 and 26.69 nm, $P < 0.05$, respectively).

Table 2 VWF and Ang-2 levels in BOECs. (Abstract VWF01)

	VWF:Ag (Mean ± SD IU/mL)		Ang-2:Ag (Mean ± SD ng/mL)	
Subject ID	BOEC Media	BOEC Lysates	BOEC Media	BOEC Lysates
10 Controls	31.20 ± 10.62	518.80 ± 175.9	6.63 ± 4.40	36.03 ± 25.17
T3-1	1.17 ± 0.21	2.95 ± 4.00	10.02 ± 2.92	29.70 ± 7.81
T3-2	2.94 ± 2.12	389.7 ± 143.6	16.64 ± 5.93	102.39 ± 17.63
T3-3	1.27 ± 0.33	29.36 ± 6.05	2.72 ± 1.90	8.35 ± 6.99
T3-4	0.43 ± 0.00	7.70 ± 0.00	-	-

Conclusions: This is the first comprehensive study of angiogenesis in type 3 VWD BOECs showing that they have increased release of the angiogenic mediator Ang-2 and altered migration.

VWF02

Inhibition of vWF secretion with novel galpha 12 N-Terminal alpha-SNAP binding domain peptide increases survival in septic mice

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Background: Severe sepsis is associated with disseminated intravascular coagulation (DIC) as a result of interdependent mechanisms of systemic inflammation, microvascular thrombosis, and thrombocytopenia. A poorly understood mechanism and yet critical determinant of sepsis-induced microvascular thrombosis is von Willebrand Factor (vWF) secretion by activated endothelial cells. We recently discovered that heterotrimeric G protein alpha subunit G12 plays a critical role in basal and evoked vWF secretion by endothelial cells by promoting Weibel-Palade body (WPB) exocytosis.

Aims: We generated a myristoylated Galpha12 N-terminal alphaSNAP binding domain blocking peptide (Myr-SBD) and tested the hypothesis that this would selectively and potently inhibit vWF secretion, limit platelet adhesion, and prevent microvascular thrombosis associated with cecal ligation and puncture (CLP) induced sepsis.

Methods:

1. CLP in mice
2. iv bolus treatment with Myr-SBD
3. plasma vWF measurement
4. survival rates

Results: CLP-induced fulminant sepsis in mice was associated with a 2-3-fold increase in plasma vWF within 24 hrs. Importantly, we observed reduced plasma vWF levels 24 hrs after CLP surgery in mice given a one-time i.v. bolus (80 μg/30 g mouse) of micellar Myr-SBD (lipid to peptide molar ratio of 90:4) at the time of surgery as compared to Myr-scrambled peptide or vehicle only group. Strikingly, this was associated with increased survival without adversely inducing hemorrhage and vascular leakage. Furthermore, and consistent with the hypothesis that Gα12-dependent increase in vWF secretion during sepsis leads to poor outcome, control WT mice succumbed to sepsis in < 96 hrs whereas 80% of $G\alpha12^{-/-}$ mice shown previously to have significantly reduced plasma vWF levels survived.

Conclusions: Inhibition of Galpha 12/alpha-SNAP dependent vWF secretion may therefore be an effective strategy for blocking microvascular thrombosis, disseminated intravascular coagulation, and death due to sepsis.

VWF03

Role of the arterial pulsatility in the modulation of von willebrand factor multimerisation in an animal model of continuous-flow left ventricular assist support

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Background: Continuous flow ventricular assist devices (CF-VAD) are mechanical pumps designed to support a failing left ventricle (LV). These CF-VADs produce a large decrease of arterial pulsatility. They also increase the shear stress forces leading to the loss of Von Willebrand factor (VWF) high molecular weight multimers (HMWM) and thus promoting bleeding. Bleeding is also modulated by the residual arterial pulsatility (AP) of the LV. We hypothesize that VWF may represent a key to understand the link between AP and the bleeding risk.

Aims: To study in a pig experimental model the role of AP in VWF multimerization regardless of the shear stress forces induced by the CF-VAD.

Methods: In vitro, we studied the proteolytic degradation of VWF HMWM induced by two CF-VADs with different maximum flow capacity (pump 1 & pump 2). We next developed a LV-VAD pig model to study the degree of HMWM-defect under different AP levels regardless of the shear stress forces induced by the CF-VAD. AP was estimated by measuring the carotid pulse pressure (pp). Blood samples were collected before and after initiation of CF-VAD at 5, 15, 30 min (min). VWF:CB/VWF:Ag ratio (expressed as a ratio vs. baseline) and HMWM (expressed as a ratio vs. baseline & as a ratio vs. standard) were used as surrogate markers of VWF functional defects.

Results: Both devices led to a significant decrease in pp in vivo ($P < 0.01$ vs. baseline 32 ± 5.9 mm Hg). AP was reduced partially

with pump 1 (pp = 20.3 ± 1.3 mm Hg) and almost totally with pump 2 (pp = 5.2 ± 3.9 mm Hg). Both pumps induced a significant degradation of HMWM (pANOVA < 0.001 et < 0.0001 respectively for pumps 1 & 2). HMWM loss after 30 min was significantly more important with pump 2 ($P < 0.05$). A same trend was observed with VWF:CB/VWF:Ag ratio ($P < 0.05$).

Conclusions: AP modulates the intensity of VWF defect in this porcine CF-VAD model. Preserving some AP might help to reduce VWF defect under CF-VAD support.

VWF04

Possible role of protein disulfide isomerase PDIA in mechanisms of von willebrand disease type 3

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Background: Von Willebrand factor (VWF) is a multimeric glycoprotein essential for platelet-dependent primary hemostasis. The biosynthesis of VWF high molecular weight multimers occurs in spatially separated steps in different cell compartments due to varying pH value requirements. We recently showed that protein disulfide isomerase PDIA1 is the protein that catalyzes VWF dimerization. PDIA1 forms disulfide bonds between the CK domains of two VWF monomers at the neutral pH of the endoplasmic reticulum (ER). The multimerization occurs in the acidic environment of the trans-Golgi apparatus by formation of inter-dimer disulfide bonds mediated by the VWF propeptide. Mutations in the VWF gene which result in quantitative loss of VWF lead to von Willebrand disease (VWD) type 3.

Aims: The aim of this study is to determine whether PDIA1 is also involved in cellular retention of VWF mutants associated with VWD type 3.

Methods: VWF mutants identified in VWD type 3 patients were transiently expressed in HEK293 cells. So far, we investigated mutants p.Val86Glu, p.Leu129Arg, p.Gly163Val, p.Trp377Cys, p.Gly525Glu, p.Trp1120Ser, p.Lys1794Glu, p.Cys2304Tyr, p.Cys2431Tyr, p.Cys2533Arg, and p.Gly2752Asp. To visualize VWF-PDIA1-association both proteins were detected by immunofluorescence.

Results: Our preliminary data indicate three different degrees of mutant-VWF-PDIA1-association:

1) Normal PDI co-localization compared to wildtype VWF,
2) Increased ER localization most likely associated with an increase in PDIA1 expression level,
3) VWF mutant induced alteration in PDIA1 localization associated with cluster formation of VWF and PDIA1 in the ER.

Conclusions: PDIA1 seems to be involved in different mechanisms leading to intracellular retention of VWD type 3 mutants. Further experiments are currently performed to quantify the observed variations in PDIA1-VWF-mutant-interaction, e.g. by co-immunoprecipitation and analysis of PDI expression levels in VWF mutant expressing cells.

VWF04A

Structure and mechanics of von willebrand factor are regulated by pH-dependent interactions in dimeric subunits

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Background: Activation of the plasma protein von Willebrand factor (VWF) for the formation of a hemostatic plug crucially depends on VWF's distinct ability to sense elevated hydrodynamic forces in the bloodstream, as found at sites of vascular injury, but also at sites of vasoconstriction, where VWF can provoke thrombosis. Recently, we showed that VWF's force response is tuned by a mechanically strong and highly specific intermonomer interaction in VWF's dimeric subunits. While VWF's conformation had been shown to be crucially affected by pH, it had not been clarified to what extent pH influences VWF's force response. Certainly, the pH in healthy blood vessels is precisely buffered to maintain a pH of 7.4, and small deviations already represent pathological conditions. However, we expect that the pH may indeed - locally - vary at sites of injury and inflammation.

Aims: We aimed to study the impact of pH on mechanics and structure of VWF dimers, which - as smallest repeating subunits of VWF - crucially underlie VWF's force response.

Methods: We performed atomic force microscopy (AFM)-based single-molecule force measurements on genetically engineered VWF dimers, composed of two monomers with different N-terminal peptide tags, thus allowing for pulling VWF specifically in its native force-sensing direction. Complementarily, we assessed the static structure of dimers by AFM imaging.

Results: We show that structure and mechanics of VWF are precisely regulated by pH-dependent interactions in dimeric subunits. Particularly, the recently discovered strong intermonomer interaction was observed with highest frequency at pH 7.4, but was essentially switched off at pH values below 6.8 (see Figure), corroborated by observations from AFM imaging.

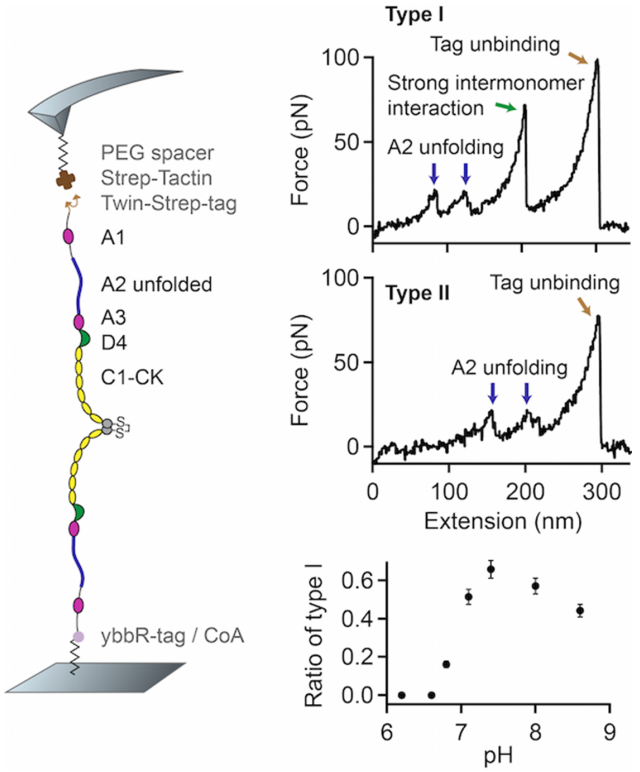

Figure Single-molecule force measurements on VWF dimers.

Conclusions: As our data suggest highest mechanical resistance of VWF at physiological pH, local deviations from physiological pH, e.g. at sites of vascular injury, may represent a smart means to enhance VWF's hemostatic activity where needed.

VWF05

Childhood-onset acquired thrombotic thrombocytopenic purpura: the french reference center for thrombotic microangiopathies experience

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Background: Thrombotic thrombocytopenic purpura (TTP) is a thrombotic microangiopathy (TMA) related to a severe functional deficiency of ADAMTS13. Acquired TTP patients exhibit an ADAMTS13 activity < 10% associated with positive anti-ADAMTS13 auto-antibodies and/or a detectable ADAMTS13 activity in remission. Acquired TTP is uncommon in children.
Aims: The aim of this study was to provide a clinical and biological picture of the first TTP boot in the French cohort of childhood-onset acquired TTP.
Methods: A cross-sectional analysis of the registry of the French Reference Center for TMAs was performed from 1999 to 2014 to identify, among childhood-onset TMA patients, those exhibiting an acquired severe ADAMTS13 deficiency at presentation. ADAMTS13 activity was measured using FRETS-VWF73 and full-length VWF ELISA; anti-ADAMTS13 IgG were titrated using TECHNOZYM® commercial kit. An exhaustive analysis of clinical records was assessed.
Results: Forty-five patients were enrolled. The age at diagnosis ranged from 1 week to 17 years old (median: 10 years old); sex ratio was 2.5F/1M. At presentation, all patients had hemolytic anemia (mean hemoglobin 7.4 g/dL) and severe thrombocytopenia (mean platelet count: 21 G/L); 26 patients had visceral ischemia (kidney $n = 18$, brain $n = 15$, liver $n = 2$, heart $n = 1$). TTP was idiopathic in 19 patients while it was associated with a clinical context in 26 patients (autoimmune disease n = 11, viral infection n = 6, other n = 9). The anti-ADAMTS13 IgG were positive in 28 patients /45 (84% and 46% in the idiopathic and secondary forms, respectively). The treatment consisted in plasmatherapy, steroids, and sometimes immunomodulating agents including, from 2004, rituximab ($n = 14$).
Conclusions: Acquired TTP in children is a very rare entity. The global picture of the inaugural TTP boot appears similar to the one of adulthood-onset TTP. Further studies will be performed to characterize the follow-up of these pediatric patients.

VWF06

Clinical-biological-genetic features of adulthood-onset thrombotic microangiopathy with severe ADAMTS13 deficiency: the french experience

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Background: Thrombotic thrombocytopenic purpura (TTP) is a thrombotic microangiopathy (TMA) related to a severe ADAMTS13 deficiency (< 10%).
Aims: The identification of the mechanisms for ADAMTS13 deficiency may impact the management of TTP patients.
Methods: We performed a cross-sectional analysis of the French national registry for TMA to identify all patients with an adulthood-onset TTP included from 1999 to 2013. ADAMTS13 activity, anti-ADAMTS13 IgG and *ADAMTS13* gene were investigated by a central laboratory. Patients' clinical data were collected for correlation with their ADAMTS13 phenotype/genotype.
Results: Among 3837 adulthood-onset TMA patients, 939 (24%) had TTP including 772 with available data and samples at presentation and during follow-up (sex ratio, 2.1 F/1M and median age, 44 y.o). The prevalence of TTP in France was 13 cases per million people. At presentation, half TTPs were associated with miscellaneous clinical situations (infections, auto-immunity, pregnancy, cancer, organ transplantation. . .) whereas half remained idiopathic. Pathophysiologically, 3 distinct forms of TTP were observed: auto-immune TTP linked to anti-ADAMTS13 IgG (75.5%), acquired TTP of unknown mechanism (22%) and inherited TTP (Upshaw-Schulman syndrome (USS)) linked to mutations of *ADAMTS13* gene (2.5%). Idiopathic TTPs were mainly auto-immune whereas non idiopathic TTPs were very heterogeneous, including a high rate of unexplained mechanisms for ADAMTS13 deficiency. Obstetrical TTPs were specifically remarkable because of their very high rate (34%) of USS.
Conclusions: As TTP clinical-biological-genetic features are strongly associated with the mechanisms of ADAMTS13 deficiency, an exhaustive characterization of ADAMTS13 in TTP patients may help clinicians select appropriate immune modulators.

VWF07

Platelet-independent binding of activated erythrocytes to von willebrand factor: a pro-active role for erythrocytes in primary hemostasis

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Background: Erythrocytes can contribute to secondary hemostasis by phosphatidylserine-supported thrombin generation. However, little is

known about their potential role in primary hemostasis. While investigating erythrocyte endothelial adhesiveness and their contribution to primary hemostasis, we observed that erythrocytes, activated by calcium influx, formed microthrombi on histamine-stimulated endothelial cells.

Aims: Our research aims to understand the composition of erythrocyte-rich microthrombi on activated endothelial cells and to investigate whether erythrocyte adhesion is mediated by von Willebrand factor.

Methods: Erythrocyte adhesion to endothelial cells was investigated using in vitro flow models with primary endothelial cells. Erythrocyte and platelet adhesion to von Willebrand factor (vWF) was analyzed with fluorescently labeled anti-vWF, anti-CD235a or anti-CD42b antibodies. The involvement of vWF was tested by a lenti-viral knockdown of endothelial vWF. The role of platelets in the formation of erythrocyte rich microthrombi was analyzed by platelet depletion or platelet-erythrocyte competition assays. Binding specificity of erythrocytes to vWF was determined in flow models with recombinant or purified protein as a substrate.

Results: Ionomycin-activated erythrocytes formed microthrombi on histamine-activated endothelial cells. Fluorescent live cell imaging showed that vWF supported these erythrocyte-rich microthrombi. Knockdown of endothelial vWF prevented erythrocyte microthrombi formation. Platelet depletion proved that erythrocyte binding to vWF was platelet independent. Erythrocyte binding was specific for vWF as little or no binding was observed to collagen type I, fibronectin, fibrinogen or fibrin.

Conclusions: Activated erythrocytes are able to bind platelet-independent to vWF and may contribute to primary hemostasis. This novel finding of pro-active hemostatic behaviour of erythrocytes could contribute to the understanding of venous thrombosis.

VWF08

TPlatelet type von willebrand disease (PT-VWD) posing diagnostic and therapeutic challenges - small case series

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Background: Despite increased worldwide awareness of PT-VWD over the last decade, many uncertainties remain around this rare platelet bleeding disorder.

Aims: To correctly identify and study new patients.

Methods: We describe 4 PT-VWD cases confirmed by genetic analysis in which either the diagnosis and/or treatment posed a unique challenge.

Results:: Case 1 presented at 45 days of life with bleeding symptoms, was misdiagnosed as ITP for 12 years but meticulous platelet mixing studies confirmed the diagnosis of PT-VWD. Peripheral blood smear showed very large platelets and platelet aggregation was impaired in response to ADP and collagen. Unexplained complicated obstetric history in the un-affected mother raised a question about the impact of the carrier status of the fetus.

Case 2 presented with thrombocytopenia at birth and was misdiagnosed as neonatal alloimmune thrombocytopenia, later misdiagnosed as Type 2BVWD and was treated with VWF concentrate (Humate P) without significant thrombocytopenia. Genetic testing confirmed PT-VWD at 1 year of age, the youngest reported patient, despite the lack of family history of PT-VWD and phenotypically normal parents.

Case 3 unexplained gestational thrombocytopenia is seen with uncommon HLA type and HLA class I IgG antibodies. Patient had an anaphylactic reaction to VWF concentrate (Fandhi) but was successfully treated with DDAVP combined with tranexamic acid with no significant thrombocytopenia.

Case 4 describes gestational thrombocytopenia in a patient with a misdiagnosis of type 2BVWD and HLA class I,II antibodies. Serial platelet counts in two consecutive pregnancies showed progressive thrombocytopenia with normal VWF levels. Platelet aggregation in response to ADP and collagen was normal. Platelet count normalized at 2.5 weeks postpartum.

Conclusions: These studies represent a record of clinical observations/interventions that help improve diagnoses/management of PT-VWD, highlight the importance of genetic testing for accurate diagnosis and serve as a valuable teaching material.

VWF09

Divergent clinical and laboratory features of acquired VWF abnormalities in patients with pulmonary hypertension vs. high shear cardiac lesions: importance of the procoagulant property of VWF multimers

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Background: Von Willebrand factor (VWF) multimer disruption has been described in severe pulmonary artery hypertension (PAH) and cardiac lesions. However, acquired bleeding is rare in PAH, and more common with cardiac lesions.

Aims: To compare the VWF profile (VWF antigen, activity, multimer distribution, and whole blood platelet function analyzer-100, PFA) in PAH in comparison to cardiac disorders in which both VWF multimer disruption and acquired bleeding are known to occur.

Methods: Seventy-two patients with group 1 PAH were compared to 357 patients with cardiac lesions (CARD). Normal subjects and patients with left ventricular assist devices were referenced to illustrate the scale of abnormalities. The relationship between VWF activity / antigen to whole blood PFA was explored, and regression lines compared for PAH and CARD by ANOVA.

Results: PAH and CARD groups were similar in age, 61 ± 12 years vs. 68 ± 15 years, and percentage anticoagulated, 24% vs. 27%. Aspirin use was more frequent in the CARD group. A history of bleeding was noted in 27% of the CARD group, but in none of the PAH patients. Both VWF antigen and activity were higher in the PAH group vs. CARD, 231 ± 122 vs. 153 ± 76 and 182 ± 95 vs. 128 ± 62, both $P < 0.0001$. However the ratio of VWF activity to antigen, suggesting impaired VWF function, was lower in the PAH patients compared to the CARD group 0.80 ± 0.10 vs. 0.85 ± 0.13, $P = 0.0002$. However, VWF multimer analysis was abnormal only in 6% of PAH patients but 55% of cardiac patients ($P < 0.0001$). Despite similar reductions in VWF activity to antigen ratio, median PFA values were perturbed less in PAH than CARD (see Figure), $P < 0.0001$.

Conclusions: VWF antigen is elevated in PAH. Despite VWF activity to antigen ratios which are frequently abnormal at < 0.80 which

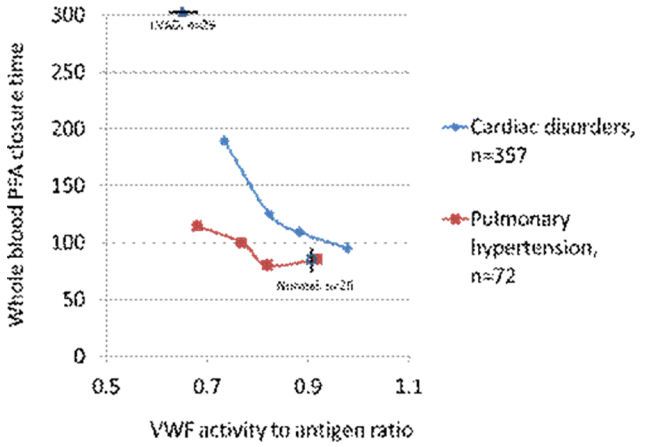

Figure (Abstract VWF09)

suggests a bleeding tendency, VWF multimers are rarely abnormal and whole blood PFA testing is only minimally abnormal compared to CARD patients. This difference in VWF properties could explain the minimal bleeding tendency in patients with PAH.

VWF10

Severe acquired von willebrand syndrome - a single centre experience

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Background: Acquired von Willebrand syndrome (AVWS) is a rare and potentially life-threatening condition.
Aims: To present the clinical observation and laboratory diagnosis of three patients with AVWS which has been treated and followed up in our Centre in the years 2007–2015.
Methods: Three patients aged 70, 61 and 30 years with no personal or family history of bleeding diathesis were examined for a sudden onset of severe bleeding symptoms. Primary diagnosis was type 3 VWD, type 2 VWD and FVIII inhibitor. Diagnosis of monoclonal gammopathy was made concurrently in two cases whereas the third patient was diagnosed with systemic lupus erythematosus. We measured FVIII, VWF: Ag, RCo, propeptide and multimers. Pharmacokinetics of plasmatic FVIII/vWF concentrate was performed.
Results: Detailed coagulation testing pointed to vWD type 3 in two patients. FVIII and VWF inhibitors were negative in one patient and a mildly positive in the second patient. Pharmacokinetics of plasmatic FVIII/vWF concentrate revealed low recovery of FVIII and no change in vWF:RCo level. vWFpp/ vWF:Ag ratio was from 8 to 300 in this two patients (normal range in type 3VWD is 0 - 1). Results of the third patient had high VWpp and VWFpp/VWF:Ag ratio and other markers pointed to VWD type 2. Thus, diagnosis of AVWS was confirmed in all the three cases. Bypassing therapy with rFVIIa was used in all the patients to treat bleeding episodes that were life threatening in one case as well as to cover invasive procedures including major surgery (total knee replacement). The effect of therapy was very good to excellent in all cases. Remission of vWF inhibitor was achieved in one case after successful treatment of SLE with combined immunosuppressive therapy.
Conclusions: Clinical and laboratory diagnosis of AVWS is difficult and it varies from case to case. vWFpp may be helpful in confirming the diagnosis. rFVIIa can be used with success to treat bleeding episode as well as to cover invasive procedures in these cases.

VWF11

COMPASS-VWF: an international multicenter study to compare VWF activity assays. Report on assay performance

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Background: Ristocetin cofactor activity (VWF:RCo), the former gold standard for measuring von Willebrand factor (VWF) activity, suffers from poor precision and sensitivity. This has led to the development of several new assays with improved characteristics. However, little is known about how these assays compare to VWF:RCo and to each other.
Aims: To compare characteristics of currently available VWF activity assays.
Methods: An independent international multicenter study was organized by the ISTH SSC VWF Subcommittee, (**Comp**arison of **Ass**ays to Measure **VWF** Activity, **COMPASS-VWF**). Eight laboratories participated worldwide. A total of 95 plasma samples were frozen, blinded, and distributed. Participating laboratories measured VWF:RCo and VWF:Ag as well as one or more of the following commercially available new VWF activity assays: VWF:GPIbR (IL HemosIL®* & IL AcuStar®*), VWF:GPIbM (Siemens INNOVANCE® VWF Ac*), as well as VWF:Ab (IL, HemosIL). Two laboratories performed in-house ELISA. Passing and Bablok regression analysis, determination of normal ranges, ROC analysis in two different settings (VWF activity in normal controls vs. patients; and specific activity, i.e. activity/Ag ratios in controls + type 1 patients vs. type 2 patients) were conducted in R package 'mcr'.
Results: All tests correlated well with the VWF:RCo, and differences are within acceptable limits. The lower limits of reference ranges were similar for all assays ranging from 36.5 to 46.1 U/dl**. Optimal cutoff in ROC analysis was found somewhat lower, 27.6 to 34.9 U/dl, yielding a specificity of 100% for all assays and sensitivities ranging 91 to 97%. ROC analysis of specific activity (i.e. activity/VWF:Ag) is shown in the Table.

Table ROC analysis of VWF specific activity (Ac/Ag).

Assay:	VWF:RCo	INNOVANCE GPIbM	AcuStar GPIbR	HemosIL GPIbR	HemosIL VWF:Ab	ELISA GPIbM
Cutoff	*0.602*	*0.678*	*0.691*	*0.647*	*0.587*	*0.611*
Sensitivity	0.867	0.867	1	0.667	0.733	0.867
Specificity	0.906	0.969	0.859	0.969	1	0.875
Pos. Pred. v.	0.684	0.867	0.625	0.833	1	0.619
Neg. Pred. v.	0.967	0.969	1	0.925	0.941	0.966

Conclusions: In general, all assays performed well and yielded results close to the (g)old standard VWF:RCo. Detailed analysis of the COMPASS-VWF study will provide the VWF community with valuable data regarding subtle differences between assay behaviors.
*Not available for sale in the US. Product availability varies by country.

** Since reference intervals vary from laboratory to laboratory, each laboratory must establish its own reference intervals.

VWF12

Functional characterization of c.4876instgc and c2555a>g VWF gene variants identified Type 2 VWD patient population in turkey

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Background: Hemostatic protein von Willebrand Factor (VWF), secreted mainly by endothelial cells, undergoes a series of post-translational modification and is released constitutively or stored for the regulated release from WPBs. Abnormalities in the biosynthesis, in the functional domains or increased clearance of plasma VWF are likely to contribute to pathogenesis of von Willebrand Disease (VWD).
Aims: To functionally characterize the VWF gene variants, novel c.4876InsTGC (exon 28) and known c.2555A>G (exon 20) identified in Type 2 VWD patients in Turkey in a heterologous cell system.
Methods: The variations were generated in a VWF cDNA expression vector by in vitro site directed mutagenesis. The effect of the variations on the intracellular localization was analyzed in HEK293 cells by immunofluorescence antibody staining and confocal microscopy. The biosynthetic effect of the variations was analyzed in COS7 cells. The wild type VWF expression vector and the VWF variant expression vectors were transiently transfected into COS7 cells alone or together to generate wild-type, mutant homozygous and heterozygous genotype, VWF:Ag levels were assayed in cell lysates and in the conditioned media using ELISA.
Results: Expression studies for the c.2555A>G substitution are currently in progress. Confocal analysis of the transfected HEK293 cells demonstrated impaired intracellular localization of c.4876InsTGC-VWF. Transient transfection of COS7 cells showed significant decrease in c.4876InsTGC-VWF secretion ($P < 0.05$, n = 3), significant increase in its intracellular levels ($P < 0.05$, n = 3) in the homozygous and heterozygous transfections relative to the secretion of wild-type VWF.
Conclusions: This study demonstrated that c.4876InsTGC, which causes Leu insertion between p.1541Leu-1542Glu in the VWF A2 domain, results in increased intracellular retention of the protein with a dominant negative effect on the processing of the wild type protein and impaired intracellular localization of c.4876InsTGC VWF in vitro.

VWF13

Inhibition of a mouse-human chimeric monoclonal antibody to von willebrand factor on VWF A3-collagen and VWF A1-platelet interactions

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Background: Collagen-VWF-platelet GPIb axis is crucial for hemostasis and thrombosis which represents a promising target for the development of new antithrombotic agents.
Aims: To develop a mouse human chimeric anti-VWF A3 antibody which blocks interactions between VWFA3-collagen and VWFA1-platelet interactions and to provide a novel antithrombotic drug candidate.
Methods: The variable cDNA sequences from hybridoma cell line, SZ123, anti-VWFA3 domain was cloned by 5'-rapid amplification of cDNA ends method. The PCR product was cloned into eukaryotic expression vector pMH3 to form fusion gene with constant regions of IgG1 heavy chain and kappa chain respectively. The two plasmids were cotransfected into CHO S cells with electroporation method and the positive clone cells were screening with 2.4 mg/L of G418. The higher expression level cell line by twice subclone was suspension cultured. The chimeric antibody in supernatant by shaking cultured was purified with protein-A column. The purified antibody was identified by SDS-PAGE. The chimeric antibody activities were identified with VWF collagen binding assay and ristocetin-induced platelet aggregation.
Results: A cell clone named MHC-SZ123 was gotten with its expression level of IgG at 200 mg/ L in the supernatants by shaking cultured. IgG purity was 95 %. SDS-PAGE shows two bands at 50 kDa and 25 kDa under reducing condition. Collagen binding assays show that MHC-SZ123 (0.125-16 ug/mL) dose-dependently blocked binding of plasma VWF from human and Rhesus monkey to human collagen type III. Platelet aggregation test shows that MHC-SZ123 at 6 and 8 ug/ml nearly 100% inhibited human and Rhesus monkey platelet aggregation induced by1.25 mg/ ml of ristocetin respectively.
Conclusions: These results demonstrated that the chimeric antibody was successfully engineered and suggested that MHC-SZ123 is a promising and more suitable therapeutic antibody by inhibiting VWFA3-collagen and VWFA1-platelet interactions for prevention and treatment of arterial thrombosis.

VWF14

Monoclonal antibody that binds to the C-terminus of the A2 domain of von willebrand factor effectively inhibits platelet adhesion to collagen in a force-dependent manner

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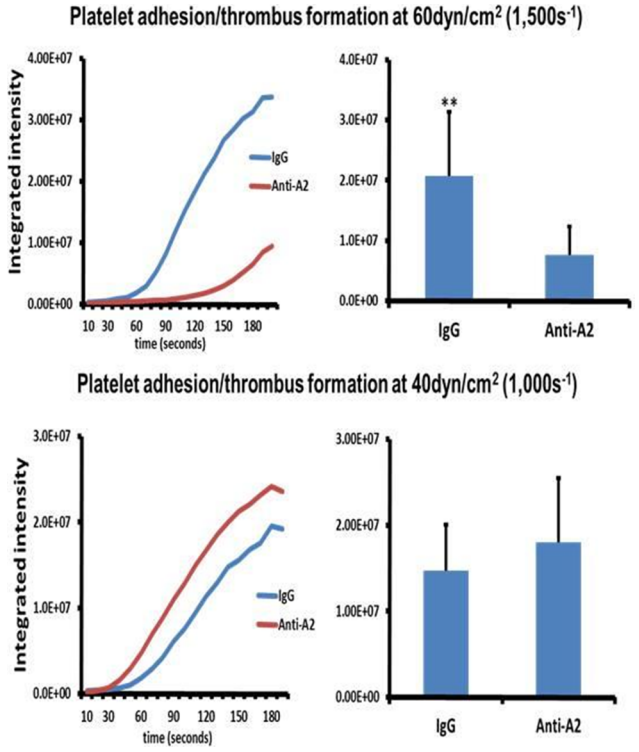
Background: Von Willebrand factor (VWF) is essential in hemostasis. The interaction between A1 and A2 domains in VWF inhibits the binding to platelet glycoprotein (GP)Ib. This autoinhibitory mechanism can be terminated when VWF senses elevated hydrodynamic forces that lead VWF to bind to platelets and collagen. The A2 domain is a force-sensing domain that regulates VWF response to shear stress. Recent studies have proposed that the force-dependent unfolding process of VWF begins from the C-terminus of the A2 domain.
Aims: We examined whether a monoclonal antibody against the C-terminal side of the cleavage site in A2 domain of VWF impacts the force-dependent unfolding of VWF, affecting platelet adhesion to collagen at different shear stress.
Methods: Whole blood from human incubated with antiA2 antibody or isotype IgG was perfused over a surface coated with collagen at shear rates of $1{,}000s^{-1}$ or $1{,}500s^{-1}$ using a microfluidic flow chamber. The antibody was also tested in ristocetin-induced platelet agglutination (RIPA) using platelet rich plasma.
Results: Platelet adhesion and microthrombi formation on collagen was significantly inhibited by the antiA2 antibody as compared to blood containing isotype IgG at shear rate of $1{,}500s^{-1}$. Unexpectedly, the antiA2 antibody did not reproduce the inhibitory effect at shear rate of $1{,}000s^{-1}$. Moreover, this antibody failed to inhibit RIPA.



Figure

Conclusions: Antibody to the C-terminus of A2 domain of VWF inhibits platelet adhesion to collagen at shear rate of $1{,}500s^{-1}$ but not at $1{,}000s^{-1}$. The inhibition is not by blocking directly the A1-GPIb interaction. We speculate that shear rates that induce peak forces ($>1{,}000s^{-1}$) differentially unfold the A2 domain, allowing the antibody to rapidly bind to its contact site. The antibody may enhance structure stability, preventing further structural changes required to increase the A1-GPIb binding. Aiming C-terminus of A2 domain could specifically reduce arterial thrombosis.

VWF15

Immunohistochemical analysis of von willebrand factor biodistribution in liver endothelial cells

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Background: Endothelial cells in the liver are one of the sites of VWF synthesis and clearance, however, the biodistribution of VWF and its subcellular localization within this organ is not well characterized. Polymorphisms in clearance receptors expressed by liver sinusoidal endothelial cells (LSECs) are associated with plasma levels of VWF.
Aims: The aims of this study are to characterize the sites of VWF synthesis and clearance in the murine and human liver.
Methods: Human plasma-derived VWF was infused into VWF-deficient mice. Immunohistochemical analysis was performed on FFPE livers from normal and VWF-deficient mice and normal humans.
Results: VWF staining was associated with the vascular and sinusoidal endothelial cell marker CD31 (PECAM-1) in normal human and murine liver tissues. VWF staining was strongest in vascular endothelial cells, and weaker and punctate in nature when associated with the liver sinusoids. In human livers, LSECs stained positive for CLEC4M and CD31; a subset of LSECs also stained positive for stabilin-2. The majority of VWF within the sinusoids was associated with CLEC4M/CD31/stabilin-2 expressing cells. VWF partially co-localized with EEA-1, a marker of early endosomes, and LAMP-2, a marker of late endosomes/lysosomes, suggesting that at least some of the VWF in the liver sinusoids is endocytosed and catabolized by these cells. VWF was also partially associated with the Weibel-Palade body marker Rab27a. In the livers of VWF deficient mice that had received plasma-derived human VWF, VWF was predominantly associated with sinusoidal CD31 and F4/80 positive cells.
Conclusions: In the murine liver, circulating human VWF is endocytosed by LSECs and Kupffer cells. In the human liver VWF is associated with LSECs expressing the endocytic clearance receptors stabilin-2 and CLEC4M. Endothelial cells in the liver likely serve to synthesize and secrete as well as internalize and degrade VWF.

VWF17

Modification of ADAMTS13 isoforms by estradiol and histamine treatment

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Background: ADAMTS13 is a physiological von Willebrand factor (VWF) cleaving protease; it is synthesized by hepatic stellate cells, megakaryocytes, endothelial cells and other cell lines. The expression of mRNA of ADAMTS13 in several organs, peripheral blood leukocytes and human platelets was demonstrated. In HEP3B was found ADAMTS13 isoforms. We described previously mRNA of isoform 1 (Iso1) and isoform 2 (Iso2) in HUVEC, platelets and two breast cancer lines (MDA-MB23, MCF7). Intracellular mRNA levels of ADAMTS13 were increased significantly in HUVEC after treatments with estradiol (E2) and Histamine (H).
Aims: Determine the effect of E2 and H on levels of ADAMTS13 Iso1 and Iso2 in HUVEC, MDA-MB23 and MCF7.
Methods: Reagents: 1 nM E2, 100 μM H, 1 mM H inhibitors (I) (HRH1: Ketotifen, HRH2: Cimetidine, HRH3: Clobenpropit).
Treatments: a) Vehicle, b) H, c) E2, d) H+E2, e) H+I, f) E2 + I, g) H+E2 + I, h) I
mRNA was extracted from cells using TRIzol reagent followed by precipitation with phenol/chloroform. The integrity was verified by 260/280 optical density ratio. The mRNA was reverse transcribed into cDNA using random primers. We designed primers to amplify the sequence of ADAMTS13 to differentiate Iso1 (610 bp) and Iso2 (442 bp) at the same time. β-actin were used as control. The PCR products were analyzed on agarose gel containing SYBR Safe.
Results: The HUVECs treated with b) showed normal level of Iso1 and with c), d), e), f), h) slight decrease of Iso1. Treatments e), f), h), also decreased Iso2; on the other hand, the HUVECs treated with b), c), d) and g) promoted increase of the Iso2. The samples were compared with a).
In MDA-MB23 and MCF7 (with initial high levels of Iso1 and Iso2 compared with HUVEC) we did not observe important differences between levels of Iso1 and Iso2 treated or not.
Conclusions: Our results show that treatment with b) and c) influence directly or indirectly in splicing mechanism modifying the levels of Iso1 and Iso2 in HUVEC, but not in MDA-MB23 and MCF7 at the concentration used.

VWF18

HemosIL VWF:RCo® werfen: a more sensitive reagent than the reference technique for the detection of acquired von willebrand disease in thrombocytosis related to myeloproliferative neoplasms

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Background: Von Willebrand factor (VWF) is composed of multimers and the highest molecular weight multimers (HMWM) carry the most important haemostastic effect. Patients with myeloproliferative neoplasms (MPN) and high platelet count (> 1500 G/l) are at risk of bleeding because of a decrease of HMWM, i.e. acquired von Willebrand disease (AVWD) suspected when VWF ristocetin cofactor activity (VWF:RCo) is decreased. The gold standard for VWF:RCo is the aggregometry assay but automated assays have been developed such as the HemosIL RCo, Werfen®.

Aims: To compare performance of HemosIL RCo, Werfen® with aggregometry in patients with thrombocytic (platelets > 450G/L) MPN (T-MPN) and in patients with secondary thrombocytosis (ST).

Methods: We studied 33 patients with MPN and 19 with ST with a mean platelets number respectively 789 ± 316 and 951 ± 311 G/L. We measured for each patient VWF:RCo by aggregometry assay and with the HemosIL RCo reagent on ACL 500 analyser, level of von Willebrand antigen (vWF:Ag Werfen®) and Factor VIII. For patients with RCo/Ag ratio ≤0,6 we suspected AVWD and studied VWF multimers distribution by electrophoresis and/or VWF binding capacity to collagen (VWF:CB).

Results: By aggregometry, the mean VWF:RCo in T-MPN and ST were respectively 96%±41 and 160 ± 69%, with RCo/Ag ratio 0.82 ± 0.22 and 0.90 ± 0.25 ($P = 0.24$). With HemosIL, the mean VWF:RCo in T-MPN and ST were respectively 51%±21 and 122 ± 55%, and RCo/Ag ratio respectively 0.43 ± 0.21 and 0.68 ± 0.17 ($P < 0.0001$). Among the patients T-MPN, 22 had RCo (aggregometry)/Ag>0.6 but RCo(HemosIL)/Ag≤0,6, compared with 6 patients with ST. Among 20 T-MPN patients with discordant results, 5 had decreased HMWM and/or VWF:CB≤0,6 vs. none among the 6 patients with a discordance in ST group. 2 T-MPN patients who had discordance but no decrease HMWM or VWF:CB, normalized RCo (HemosIL)/Ag when platelet number normalized under treatment.

Conclusions: The HemosIL RCo reagent seems to be more sensitive to a change in the profile of HMWF in thrombocytic MPN patients.

VWF19

Impact of platelet reactivity on individual thrombin generation in type 3 and 2 von willebrand disease: results from a small prospective study

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Background: The heterogeneity of von Willebrand disease (VWD) is remarkable and von Willebrand factor (VWF) or factor VIII (FVIII) levels do not directly predict the patient's bleeding risk.

Aims: We studied the individual thrombin generation (TG) and platelet activation in relation to the clinical phenotype in severe VWD.

Methods: Ten patients with type 3 (VWD3) and 5 with type 2 (VWD2) were studied. Rotational thromboelastometry (ROTEM: INTEM, EXTEM) was measured. TG was assessed with calibrated automated thrombogram (CAT) in platelet-rich (PRP) and -poor plasma (PPP) with 1pM and 5pM tissue factor (TF). In 6 VWD3 patients on VWF prophylaxis (PRO), baseline TG was measured after ≥72 h washout of the concentrate (dose 16-95 IU/kg) and recovery TG at 30 min. Platelet reactivity was analyzed through expression of Annexin V and P-selectin after stimulation with ADP and collagen-related peptide.

Results: ROTEM was completely normal. Baseline TG varied markedly between individuals at similar VWF and FVIII activities both in PPP and PRP (up to 15- and 8-fold). The extent of variation and reduction of TG were greatest in PPP of VWD3 detected with 1pM TF in CAT. At trough levels, VWD3 patients on on-demand (OD) with a less severe bleeding phenotype generated less thrombin than patients on prophylaxis ($P = 0.009$), even at VWF/FVIII levels < 5%. The increase in peak TG correlated tightly with the recovery of VWF and FVIII activities in PPP, but not in PRP. Annexin V and P-selectin expressed 3-fold individual variation. Intriguingly, peak TG and endogenous thrombin potential in PPP associated negatively with both P-selectin and Annexin V expression (r = -0.74, -0.88; $P = 0.0008$, 0.002). Historical bleeding score did not associate with individual TG and platelet reactivity.

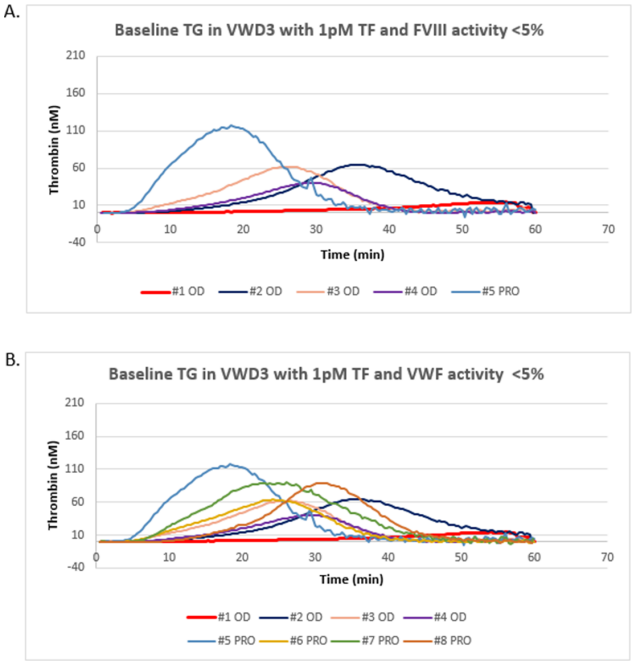

Figure Individual variation of baseline TG in PRP.

Conclusions: Standard ROTEM fails to recognize either VWD2 or VWD3. In VWD TG does not associate with low VWF and FVIII activities. Platelets and their procoagulant characteristics seem to regulate individual TG, revealed at low VWF/FVIII level and TF stimulation.

VWF20

Clinical evaluation of a new collagen-like protein with von willebrand factor binding activity: toward a New VWF: CBA

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Background: The diagnosis of von Willebrand disease (vWD) requires a panel of biological tests, often targeted to platelets / GPIb. Another approach is to measure the binding of von Willebrand factor (vWF) to collagen, but collagen binding assay (CBA) is not currently used in daily practice largely due to lack of automated assay and discrepancy regarding the source of collagen (type I and/or type III). Short recombinant collagen engineered to bind to vWF with well defined binding sites could represent an alternative to native collagens.
Aims: To evaluate the performance of a new recombinant collagen-like protein (r-NVH020B) with vWF binding activity in vWD diagnosis.
Methods: In a multicentric study, we compared an in-house Elisa CBA using r-NVH020B with 2 commercial kits (Asserachrom CBA Stago and Technozym CBA Cryopep). We included controls (n = 31) and selected patients with type 1 (n = 46), type 2 (n = 122), type 3 vWD (n = 10) according to the CRMW (Centre de Référence de la Maladie de Willebrand) criteria, acquired deficiency (n = 7) or mild deficiency (n = 50). All the type 2 vWD patients were genotyped. vWF RCo activity was performed in aggregometry.
Results: At first, r-NVH020B binding to purified vWF was demonstrated. VWD patients type 1 and type 3, patients with acquired Willebrand and controls were well identified by this in-house CBA, such as commercial CBA. For type 2 vWD (n = 122), the deficiency was properly diagnosed in patients with sub-types 2A/IIA and 2A/IIE but some differences were observed between the kits for patients with sub-types 2B, 2M and 2UC. Results with r-NVH020B CBA were strongly correlated with Stago CBA (r-pearson = 0.94, rho-lin = 0.93).
Conclusions: The performance of r-NVH020B to diagnose vWD was confirmed whatever the type of deficiency. The next step will be to automate the assay; in this respect the short size of r-NVH020B may represent a major advance.

VWF21

Recombinant von willebrand factor kinetics and ultralarge multimer content in severe von willebrand disease patients

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Background: Awareness of the highly hemostatic ultralarge multimer (ULM) content and associated kinetics of von Willebrand factor (VWF) concentrates is essential for effective and safe bleed management in von Willebrand disease (VWD). A pure recombinant VWF (BAX 111, VONVENDI) has been developed which retains a higher ratio of intact ULM than plasma-derived VWF concentrates.
Aims: Evaluate pharmacokinetics (PK) and ULM content of BAX 111 across a wide range of doses in severe VWD patients.
Methods: In two prospective trials (Phase 1 and Phase 3), BAX 111 ULM content and PK were evaluated in a total of 56 VWD patients (largely type 3 VWD) in a non-bleeding state over 96 h. Lower doses (2, 7.5, 20 IU VWF:RCo/kg) in the Phase 1 first-in-human trial were administered together with rFVIII; PK at 50 IU VWF:RCo/kg was evaluated with and without rFVIII in a crossover design; 80 IU VWF:RCo/kg PK was repeated after 6 months of on-demand bleed treatment in the Phase 3 trial.
Results: An immediate rise in ULM was followed by a marked decrease between 12-24 h presumably due to ADAMTS13-mediated proteolysis and returning to near-baseline levels by 96 h post-infusion. Similarly, VWF:RCo increased rapidly and declined along with ULM content. Across a wide range of doses (20, 50, 80 IU) there was a tendency toward a longer terminal half-life for BAX 111 (mean $T_{1/2}$ 19.1 h - 21.9 h) compared to pdVWF, with no apparent dose-dependency. Enhanced stabilization of FVIII with BAX 111 was confirmed with a secondary rise in FVIII activity 12 h post-infusion, irrespective of administration with or without rFVIII. No thromboembolic events occurred throughout repeated administration for bleed treatment in the Phase 3 study.
Conclusions: Enhanced FVIII stabilization due to the high ULM content in BAX 111 suggests a potential for dosing independent of FVIII after the first infusion. The observed susceptibility of ULM to physiologic regulation is consistent with the lack of thromboembolic events in the clinical trials.

VWF22

Phenotype analysis associated to p.Arg1315Cys (p.R1315C); the value of DDAVP and VWFpp/VWF:Ag ratio to reach the diagnosis

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Background: The p.R1315C has been described associated to 1C-VWD, 1-VWD, 2A-VWD, 2M-VWD, and 3-VWD phenotypes. Additional information can be derived from the DDAVP response and VWFpp/VWF:Ag (PP ratio).
Aims: To show our experience in reaching the definitive diagnosis in 8 patients (pt) carrying heterozygous p.R1315C, with moderate-to-severe hemorrhagic events and a previous laboratory profile suggesting 2M-VWD.
Methods: Studies before and after DDAVP infusion: FVIII:C (one-stage method) (nr 50-150 IU/dL), VWF:Ag (Ag) (nr 50-150 IU/dL) and VWFpp (PP) (ELISA), VWF:RCo (RCo) (nr 50-150 IU/dL) (aggregometric method). Bleeding score (BS) (ISTH), RCo/Ag (nr>0.6) and PP ratio (nr 0.9-2.14) were calculated. DDAVP response was tested in 6 pts and PP ratio in 8.
Results: Pt 1 and 2: Ag and RCo did not reach normal levels (NL); RCo/Ag>0.6; high PP ratio. Pt 3: Ag and RCo reached NL; RCo/Ag>0.6; high PP ratio. Pt 4 and 5: high PP ratio. **Diagnosis: 1C-VWD.**
Pt 6:Ag and RCo did not reach NL; RCo/Ag>0.6; normal PP ratio. **Diagnosis: severe 1-VWD.**
Pt 7:Ag and RCo reached NL; RCo/Ag>0.6; normal PP ratio. **Diagnosis:** type **1-VWD.**

Pt 8:Ag did not reach NL; RCo did not change; RCo/Ag< 0.6; normal PP ratio. Initial **2M-VWD: confirmed.**

Conclusions: Both DDAVP response and PP ratio were useful to reach the diagnosis in our 8 pt.

p.R1315C was associated to 1C-VWD in 5/8 pt (62.5%), severe type-VWD in 1 pt, type 1-VWD in 1 pt, 2M in 1 pt (12.5% each).

We found different phenotypes associated to p.R1315C, mainly 1C-VWD.

However, it should not be discarded the presence of other mutations in the *VWF* gene, which together with p.R1315C would result in the different phenotypes found in pt.

Table DDAVP response and PP ratio in patients. (Abstract VWF22)

Pt	BS	FVIII:C pre- post	Ag pre- post	RCo pre- post	RCo/Ag pre- post	PP ratio	Diagnosis
1	3	28- 45	20- 24	<10- 16	0.4- 0.66	2.6	1C-VWD
2	3	35- 50	9- 25	<10- 24	1.0- 0.9	3.3	1C-VWD
3	1	20- 140	23- 57	<10- 59	0.4- 1.0	2.8	1C-VWD
4	6	45-	23-	<10-	0.4- -	2.3	1C-VWD
5	4	37-	12-	<10-	0.7- -	4.96	1C-VWD
6	5	35- 80	8- 28	<10- 18	1.0- 0.64	1.6	Severe 1-VWD
7	5	35- 95	36- 70	<10- 66	0.3- 0.9	2.0	Type 1-VWD
8	3	25- 50	15- 41	<10- <10	0.1- 0.2	1.3	2M-VWD

VWF23

A comparative evaluation of a new fully-automated assay for von willebrand factor collagen binding activity to an established method

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Background: von Willebrand factor (VWF) is a multimeric plasma glycoprotein that acts as a mediator of platelet adhesion to subendothelial collagen at the site of vascular injury and as a carrier for coagulation factor VIII in plasma. Laboratory diagnosis of von Willebrand disease (VWD) is made by measurement of both VWF protein level and activities. Current VWF activities tests include ristocetin cofactor and collagen binding (VWF:CB) assays.

Aims: We have undertaken an evaluation of a new, fully-automated, chemiluminescent, VWF:CB assay relative to an established enzyme-linked immunosorbent assay (ELISA) method.

Methods: We used two analytical systems that operated with different detection principles: a colorimetric ELISA by Asserachrom Stago (type III collagen from human placenta) and a chemiluminescent method performed on ACL AcuStar Analyzer (Instrumentation Laboratory, US). The new HemosIL AcuStar VWF:CB assay is a chemiluminescent 2-step immunoassay that uses magnetic particles coated with a type III collagen-triple-helical peptide. VWF:CB levels were determined in 50 normal subjects and 100 VWD patients (22 type 1, 73 type 2 and 5 type 3).

Results: All VWD type 3 samples reported values were below the lower detection limit with both methods (1 IU dL^{-1}). The new method showed a good correlation with ELISA ($r > 0.9$, mean bias -3.85) in both normal and VWD samples (Figure). Two out of 150 samples gave inconsistent results between the two assays.

Conclusions: The new assay is rapid and simple to use, with its ready-to-use reagent cartridges. The VWF:CB assay, in addiction to HemosIL AcuStar VWF:Ag and VWF:RCo assays, makes much easier the diagnosis of VWD in both non-specialized and reference laboratories. However, the assays results should be always integrated in association with the clinical information of patients, in forming a diagnosis.

VWF24

Von willebrand factor (VWF) assays and the diagnosis of von willebrand disease (VWD). Who follows the guidelines?

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Background: In 2014, UKHCDO published guidelines on VWD (*Laffan et al, Br J Haematol 2014; 167: 453-465*).

Aims: To assess current practice in diagnosis and classification of VWD.

Methods: a questionnaire was sent in May 2015 to UK NEQAS BC participants; responses were recorded as described below.

Results: Of 96 centres performing VWF assays, 63 (66%) used VWF antigen and activity assays. A further 28 (29%) also employ a collagen binding assay (VWF:CB); no centre performs VWF:CB without VWF activity assays. *The guidelines recommend measurement of FVIII, VWF:Ag and VWF activity for VWD diagnosis with VWF activity assessed via binding to both GPIb and collagen,* an approach adopted by just 28/96 centres. Assays based on monoclonal antibodies against the VWF GPIb-binding site are not recommended, but are used by 23% of centres.

For diagnosis, 9 centres reported < 30u/dl VWF activity, with a bleeding history, as diagnostic of VWD. 33 centres employed reference ranges for diagnosis, and 12 used blood group-specific ranges. Others used diagnostic criteria of VWF < 40 or < 50u/dl. *The guidelines recommend VWD can be diagnosed when VWF activity is < 30iu/dl, and that reference ranges are not employed.* Over 75% of centres do not follow this recommendation.

Approaches for subtyping VWD included additional testing (RIPA, Multimers, FVIII binding assays and genetic analysis). VWF activity/antigen ratio was employed by 48%, with 0.6 or 0.7 as the cut-off to separate types 1 and 2 VWD. *The guidelines recommend classification includes an activity/antigen ratio cut off of 0.6, RIPA assays on all samples with ratios below this, and multimer analysis or both VWF:RCo and VWF:CB: to VWF Ag ratios*

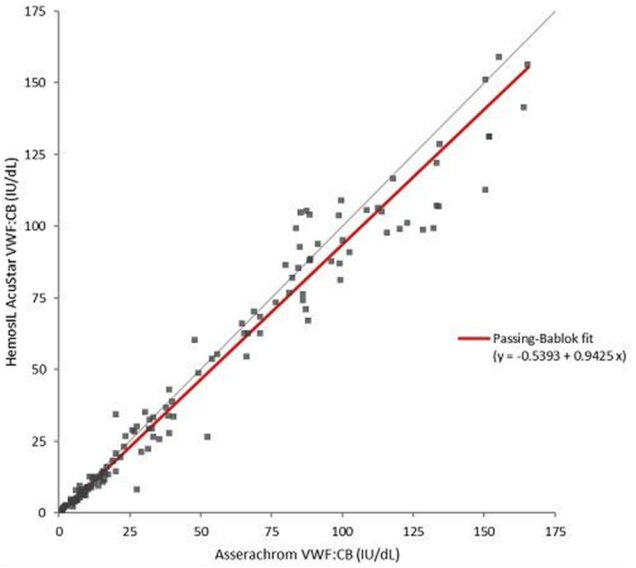

Figure Method Comparison (after repetitions) – P.

to discriminate 2A and 2M VWD. No centre reported use of such ratios, and just 30% reported availability of multimers. Thus < 1/3 of centres followed the classification recommendations.
Conclusions: This report shows widespread lack of uptake of UK HCDO recommendations >6 months after publication.

VWF25

Computer modeling of platelet adhesion to a surface with grafted von willebrand factor multimers

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Background: The mechanics of platelet initial adhesion due to interactions between GPIb receptor with von Willebrand factor (vWf) multimers is essential for thrombus growth and the regulation of this process. Multimeric structure of vWf is known to make adhesion sensitive to the hydrodynamic conditions, providing intensive platelet aggregation in bulk fluid for high shear rates [Soft Matter, 2013, 9, 10381]. But it is still unclear how it affects the dynamics of platelet motion near vessel walls and efficiency of their adhesion to surfaces.
Aims: Our goal is to resolve the principal issues in the mechanics of platelet initial attachment via GPIb-vWf bonds in near-wall flow conditions: when the platelet tends to roll or slide and how this dynamics depends on the size, conformation and adhesive properties of the vWf multimers.
Methods: We develop a computer model based on a combination of the Lattice Boltzmann method for calculating hydrodynamics with mesoscopic particle dynamics method for simulation of vWf multimers. The adhesive interactions between vWf and platelet surface are modeled via tunable Morse potential.
Results: As the computer simulations were performed, we found that the increasing shear rate leads to the spreading of vWf over the surface. This implies that shear provides the increase of adhesion sites density on a surface, rather than the effective distance of platelet capture. We also provided the parameter maps for the regimes of platelet adhesion dynamics.
Conclusions: Our results reveal the link between the mechanics of platelet initial adhesion and the physico-chemical properties of vWf multimers. This has implications in further theoretical investigation of thrombus growth dynamics, as well as the interpretation of in vitro experimental data. The reported study was funded by RFBR according to the research project №16-31-60061_mol_a_dk and by the Stipend of President of Russian Federation for young scientists.

VWF26

Effects of bilirubin on ADAMTS-13 activity measured with a FRETS rVWF73 substrate-based fluorescent assay

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Background: Accurate measurement of plasma ADAMTS-13 activity is crucial for the diagnosis and follow-up of TTP patients. Plasma bilirubin is suspected to interfere with the FRETS-VWF73-based ADAMTS-13 activity assays, by two distinct mechanisms: a direct inhibitory effect of bilirubin on the enzyme activity and a fluorescence quenching effect.
Aims: Investigate the impact of bilirubin on the measurement of ADAMTS-13 activity using FRETS-VWF73.
Methods: Citrated plasmas from healthy volunteers (HV pool) and icteric plasmas from 19 patients with thrombotic microangiopathy (IPP) were incubated in various conditions with unconjugated bilirubin (UB; 0-500 μM) and/or bilirubin oxydase (BO: 2 U/mL). ADAMTS-13 activity was measured by incubating plasmas diluted 1:20 and 1:40 in 5 mM Bis-Tris, 25 mM CaCl2, 0,005% Tween 20, pH 6,0, with 0.7 μM FRETS-VWF73, and by recording fluorescence for 1 h at 30°C (Ex: 340 nm; Em: 450 nm, kinetics analysis). The quenching effect of UB was assessed on the HV pool autofluorescence.
Results: UB quenched the autofluorescence of HV pool by 55% at 500 μM and had no significant effect below 31,25 μM. The quenching was strongly reverted by addition of BO. UB partially inhibited ADAMTS-13 activity of HV pool by 27.3% at 500 μM (7.4% at 31,25 μM). Incubation of IPPs (total bilirubin ranging from 4.8 to 348.4 μM) with 2 U/mL BO strongly decreased total and direct plasma bilirubin concentrations, but did not significantly increased ADAMTS-13 activity (+3.2 ±14.3%, P=0.365). No association was found between the % decrease of bilirubin by BO and the variation of ADAMTS-13 activity (r^2=0.1351, NS).
Conclusions: in our clinical laboratory settings - i.e. plasma diluted 1:20 - the impact of plasma bilirubin quenching on ADAMTS-13 activity measurement with FRETS-VWF73 is negligible. Purified UB partially inhibited ADAMTS-13 activity. However, we could not demonstrate any significant direct inhibition of ADAMTS-13 activity by plasma bilirubin through the use of BO.

VWF27

Calibration with the 1st international standard for ADAMTS-13 Using Technozym® ADAMTS-13 activity assay: patient correlation data

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Background: Determination of ADAMTS-13 activity levels provides a valuable tool for diagnosis and patient follow-up in TTP and aHUS. Accurate and precise assays are required especially in the low range of decision. Recently the 1st International Standard for ADAMTS-13 has been established.
Aims: In the present study we evaluate the TECHNOZYM® ADAMTS-13 activity ELISA calibrated against the 1st IS for ADAMTS-13 in plasma and correlate results between calibration in % of normal and IU/mL.
Methods: The activity measurement of this test is based on detecting cleavage of a GST-labeled vWF73 substrate bound to the plate by means of a monoclonal antibody (conjugated to HRP) specific for the cleavage site. The assay use a set of 6 standards, prepared from normal plasma diluted into ADAMTS-13 depleted plasma. These standards were calibrated against the 1st IS for ADAMTS-13 in IU/mL or against a calibrator set, calibrated against an inhouse standard plasma pool and expressed in % of normal. Patient samples were citrated plasma.
Results: Within run reproducibility was assayed using 8 samples with different ADAMTS-13 activity levels and measured in 10 replicates. All samples reported CVs between 5.1% and 9.2 %. Inter-assay variation most important in the low range of decision was 8.8% for the sample with 0.16U/mL and 9.8% for the sample with 0.07U/mL.
Linearity of dilution showed R square of 0.998 and 1/Slope of 1.007 for all tested patient samples.

The correlation of results determined in % of normal and in IU/mL showed R square of 0.998 with a slope of 0.0096 and intercept of 0.0125.

Conclusions: We are reporting comparison and validation of ADAMTS-13 Activity ELISA after calibration against the 1st IS for ADAMTS-13. ADAMTS-13 Activity levels levels can be determined with high precision after calibration in IU/mL. Due to the excellent correlation of results normal ranges for ADAMTS-13 activity and patient results determined in % of normal can easily be recalculated as 100% = 1 IU/mL.

VWF28

Ferritin promotes secretion of von willebrand factor (VWF) from endothelial cells: a potential mechanistic implication for patients with pathological immune activation

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Background: Hemophagocytic Lymphohistiocytosis (HLH) is a pathologic immune-activation syndrome associated with high mortality due to cytokine storm, disseminated microvascular thromboses, and unresolved infection. Elevated ferritin, composed of heavy (FHC) and light chains (FLC), is one of the diagnostic criteria for HLH. Many patients with HLH have ferritin levels > 100 000 ng/mL, with levels > 10 000 ng/ml associated with mortality. Although ferritin has been extensively studied in iron metabolism, it can also initiate cellular signaling. We have reported that autopsies from 50% of HLH patients demonstrated disseminated microvascular thromboses which can be composed of fibrin or VWF-rich microthrombi. VWF can be secreted from endothelial cells. It is unknown whether high levels of ferritin in HLH patients actively participates in disease progression.

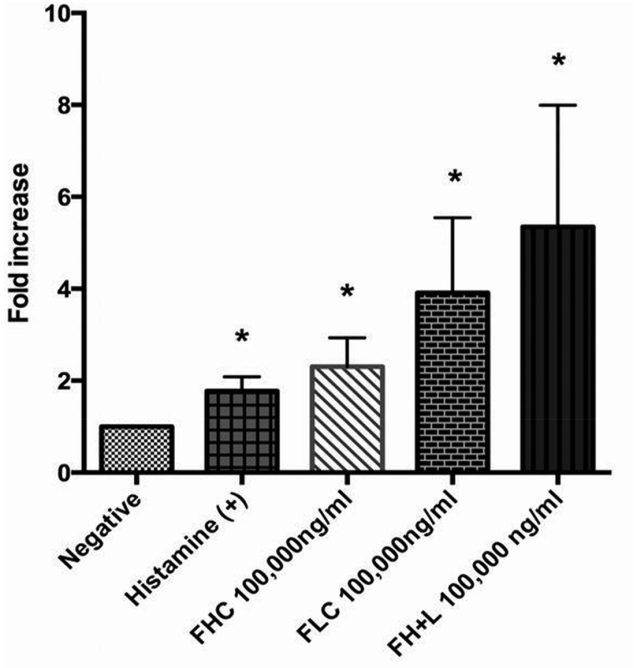

Figure 1: Ferritin Heavy Chain (FHC), Ferritin Light Chain (FLC) and Ferritin Heavy + Light Chain (FH+L) significantly increases VWF release from HUVEC. *p<0.05 as compared to negative control.

Aims: To test whether FHC and FLC can facilitate the secretion of VWF from endothelial cells.

Methods: Human umbilical vein endothelial cells (HUVEC) were stimulated with 100,000 ng/ml of recombinant FHC, FLC, or a combination (FHC+FLC). Secreted VWF was measured from media by ELISA. HUVEC lysate was separated on SDS PAGE gel and immunoblotted with anti-phospho PKC a Thr ^{638} and anti-actin (loading control) antibodies.

Results: Compared to the control (unstimulated) HUVEC, recombinant ferritin significantly increased VWF secretion. The highest increase was with FHC+FLC (5-fold; $P< 0.05$), followed by FLC (4-fold; $P< 0.05$) and FHC (2-fold; $P< 0.05$). Consistent with reports that agonist treatment increased PKC activation and promoted VWF secretion, we noticed that phosphorylation of PKC a Thr ^{638} was moderately increased in HUVECs treated with FHC, FLC and FHC+FLC.

Conclusions: Ferritin at concentrations seen in HLH patients promoted significant increases in VWF secretion *in vitro*, with the combination of FHC+FLC inducing a synergistic effect. These observations may support the notion that ferritin activates the hemostatic pathway in patients with HLH.

VWF29

Understanding "normal": baseline VWF characteristics in normal BOECs

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Background: Blood outgrowth endothelial cells (BOECs) are a relatively new model used for studying von Willebrand disease (VWD) *ex vivo*. BOECs are obtained via a blood sample, express endothelial cell markers, and contain Weibel-Palade bodies (WPBs) making them an ideal model for studying VWD pathogenesis.

Aims: To isolate BOECs from healthy donors to obtain baseline von Willebrand factor (VWF) data to use as a normal range when studying VWF processes, including angiogenesis.

Methods: Healthy controls were recruited; bleeding scores, plasma VWF/FVIII levels, ABO, *VWF* genotype were obtained and blood was collected for BOEC isolation. Secreted and cellular VWF and angiopoietin-2 (Ang-2) from BOECs was measured and storage of VWF and Ang-2 observed using immunofluorescence. Proliferative capacity, migration, and tubule formation of BOECs was measured. Queen's University provided ethics approval and consent was obtained.

Results: Twenty-nine individuals were enrolled. 38 isolations, including repeated isolations in the same individuals, were performed with 68% success. Data from 14 individual BOEC lines (9 females, 5 males; mean age=36 yrs, range 17–62 years) are presented. Table 1 shows plasma VWF/FVIII levels and VWF/Ang-2 levels in media and lysates of BOECs. Overall plasma VWF:Ag correlated with VWF secreted from BOECs (R^2=0.176, P =0.007). VWF was stored in rod-shaped WPBs; Ang-2 co-localized with VWF however the degree of co-localization varied (mean tM coefficient=37%; range= 8.5–58%) between BOECs. Cell proliferation was also variable with a mean 8-fold increase in proliferation 144 h post seeding (range=4 to 10-fold increase). Overall, migration of BOECs was similar but tubule formation in Matrigel was highly variable and was the greatest in 4 females.

Table 1

	Plasma	BOEC media	BOEC lysates
Mean VWF:Ag, IU/mL (range)	0.99 (0.61–1.62)	–	–
Mean VWFpp, IU/mL (range)	1.08 (0.79–1.80)	–	–
Mean VWF:RCo, IU/mL (range)	0.94 (0.52–1.46	–	–
Mean FVIII:C, IU/mL (range)	1.30 (0.79–1.95)	–	–
Mean VWF:Ag, mU/mL (range)	–	31 (11–51)	653 (353–1092)
Mean Ang–2, ng/mL (range)	–	6.7 (2.4–16.3)	37 (5.2–88)

Conclusions: While BOECs provide a useful model for studying VWD pathogenesis we show a great deal of inter-individual variability even amongst normal subjects which may complicate understanding of certain cellular processes.

VWF30

ADAMTS13 in chronic liver diseases: data from the developing world

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Background: Profound hemostatic defects are noted in patients with chronic liver diseases (CLD) leading to bleeding or thrombosis. ADAMTS13, a von Willebrand factor (vWF) cleaving protease is also synthesized in liver, absence of which may lead to accumulation of unusually large VWF multimers causing thrombotic/thrombocytopenic complications.

Aims: To determine ADAMTS13 antigen (Ag) and vWF Ag levels in patients with CLD and acute on chronic liver failure (ACLF) in comparison to controls and to correlate with bleeding tendencies and mortality in these groups.

Methods: A prospective study approved by Institutional ethics board was carried out in 62 adult patients of ACLF (gp1) and CLD (gp2) respectively and 40 controls from normal healthy population (gp3). ADAMTS13 Ag and vWF Ag in all groups were determined on citrated plasma samples by using sandwich ELISA technique. Comparative analysis was done by ANOVA and subsequently by post HOC analysis (Bonferroni test). The Ag levels were correlated with bleeding and mortality rates using Pearson's correlation. P values considered significant if < 0.05.

Results: The mean ages were: 42.2±13.1, 47.04±13.8 and 35±10.1 years in gp1, 2 and3 respectively. There were 11.3% bleeders and 19.4%mortality in gp1 while 22.6%bleeders and 1.6%mortality in gp2. The mean ADAMTS13 value in gp1 (172.9 IU/L) and gp2 (145.9 IU/L) was significantly low as compared to gp3 (226.7 IU/L); P =.001. While vWF Ag value in gp1 (523.4 IU/L) and gp2 (539.6 IU/L) was significantly high as compared to gp3 (166.7 IU/L, P =0.0001). In gp1, ADAMTS13 levels were significantly higher in non bleeders (P =0.021) while in gp2 the difference was not significant. No significant correlations were observed between ADAMTS13 levels and MELD scores/mortality rates in gp1 and 2.

Conclusions: ADAMTS13 levels decline significantly in CLDs and may predict bleeding tendencies in patients with ACLF.

VWF31

Bleeding score in type 1 von willebrand [VWD] patients using the ISTH-BAT questionnaire

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Background: Challenges in assessment of the severity of bleeding symptom led to the evolution of bleeding assessment tools and in 2010, the International Society on Thrombosis and Hemostasis - Bleeding Assessment Tool (ISTH-BAT) was developed and validated.

Aims: To administer ISTH-BAT bleeding score questionnaire to the Omani type 1 vWD patients and correlate it with the clinical phenotype.

Methods: 28 Type I vWD index cases diagnosed using the ISTH-SSC criteria were personally interviewed and administered the ISTH-BAT bleeding score questionnaire. The bleeding score [BS] was calculated based on a presence or absence of the bleeding symptoms from 12 different sites according to the standard ISTH-BAT questionnaire.

Results: The mean time to administer this questionnaire was 12 min. The mean BS amongst females was 7.1, whereas, in males it was 5.2 and in children it was 4.2. Although 7% of these patients had a BS of 0, 53% [n=8] of the females, 71% [n=5] of the males and 50% [n=3] of children were above the mean normal BS cut-off of 6, 4 and 3 respectively. The BS was negatively correlated with FVIII:C levels, vWF:Ag, vWF:RiCoF and vWF:CBA respectively and the Pearson's correlation coefficient was respectively -0.29,-0.33,-0.28 and -0.43;p >0.05.

Conclusions: The ISTH-BAT BS designed to reflect the severity of bleeding demonstrated the inherent variability in the bleeding pattern. Although the mean BS was abnormal, it did not correlate significantly with the surrogate laboratory parameters used in the diagnosis of vWD.

VWF32

Correlation of phenotypic and genotypic expression of VWD type I and II in Pakistani Patients

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Background: von Willebrand disease is a common inherited bleeding disorder which is caused either by quantitative or functional defects of plasma glycoprotein von Willebrand factor (VWF). VWD is classified in to three primary types (quantitative types 1 and 3, and qualitative type2) based oncoagulation factor assays.

Aims: In this study we aimed to further characterize the subtypes of patients diagnosed with VWD on the basis of phenotype history, hemostatic investigation, VWF multimer analysis and direct gene sequencing.

Methods: We calculated bleeding score on the basis of a condensed MCMDM-1 VWD questionnaire.Initial standard assay including platelet count, F.VIII: C, VWF: Ag, VWF: Ricof and binding to Glycoprotein Ib were investigated. Multimer analysis of secreted plasma VWF was performed on 1.6% (w/v) medium resolution SDS-agarose gel and VWF gene sequenced by Sanger using big terminator cycle kit v3.1 on genetic analyzer 3130 (*Applied Biosystems*).
Results: We studied 9 index patients(IP), 2(22.2%) type I, 4(44%) II and 3(33.3%) unclassified. In type I, II and unclassified the VWF:Ag levels were ranging from 20.8 and 49.5 in type 1,26.6,66.2,68.6 &68.6 in type II and 64.9, 57.3 and 41.4 IU/dl in unclassified, VWF RCo:was 19 and 20 type 1,11,8,10 and 43 in type II and 33.4,43 and 40 IU/dl unclassified. VWF Ag/ Ricof and FVIII were also measured.Gp Ib: 17.9 and 36.6 in type 1, 4,23.1,18.2and33.9 in typeII and 48.8,34 and 36.6 in unclassified . Significant loss of large multimers in two IPS of type II and normal pattern in type I. we identified mutations in 5 IPs out of 9 IPs which were one gene conversion, 2 novel and 2 reported missense mutations.
Conclusions: Gene sequencing is the only tool for confirmation and correlation with phenotypic expression of the types and subtypes of VWD. We did not find mutation in 4 samples due to low yield of DNA.In silico modelling of the novel missense variation is required to predict their pathogenicity.

VWF33

Comparison between VWF:RCo and VWF:CB in a 2B VWD cohort

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Background: In type 2B von Willebrand disease (2BVWD) the von Willebrand factor has increased affinity for platelet glycoprotein Ibα, due to the presence of mutations in the A1 domain. The loss of high molecular weight multimers (HMWM), VWF:RCo/VWF:Ag ratio (RCo/Ag) >0.6 are frequent findings.
Aims: To compare VWF:RCo and VWF:CB, RCo/Ag and VWF:CB/VWF:Ag (CB/Ag) in a group of 2BVWD patients (pts) (n=23). Four pts were diagnosed as atypical 2B VWD (normal multimeric profile and platelet count) (p.P1266L and pM1304V), and 19 as typical 2BVWD (absent HMWM) (p.R1306W, p.R1308C, p.S13010F, p.V1316M).
Methods: VWF:RCo was assayed by aggregometry using fixed-washed platelets; VWF:CB was assayed by ELISA (collagen type 1).
Results: Atypical 2BVWD: VWF:RCo=34±17.6 IU/dL, RCo/Ag=1.01±0.2. Pts with normal RCo/Ag=100%. VWF:CB=31.2±16.3 IU/dL, CB/Ag=0.95±0.4. Pts with normal CB/Ag (>0.6)=100%. VWF:CB and CB/Ag were lower than VWF:RCo and RCo/Ag respectively, but without significant difference (P =0.8 and 0.9).
Typical 2BVWD: VWF:RCo=32.3±28.1 IU/dL, RCo/Ag=0.58±0.3. Pts with normal RCo/Ag=36.8%. VWF:CB=18.9±17.5 IU/dL, CB/Ag=0.35±0.17. Pts with normal CB/Ag=10.5%.
VWF:CB was lower than VWF:RCo, but without significant difference (P =0.08). CB/Ag was significant lower than RCo/Ag (P =0.006). Percentage of pts with normal CB/Ag was lower than those pts with normal RCo/Ag but not significant (P =0.12). Statistical difference was found in CB/Ag between atypical vs typical pts (P =0.000).
Conclusions: VWF:CB and CB/Ag were found lower than VWF:RCo and RCo/Ag respectively. However, the differences were not statistically significant, but they became evident within those patients with absent HMWM. VWF:RCo levels would be overestimated in 2B VWD, due to this known gain-of-function of VWF-mutant in A1-domain, whereas VWF:CB evaluates VWF-collagen binding and is impaired by VWF-mutants in A3-domain, and also the absence of HMWM. We think that difference in CB/Ag between atypical and typical pts is closely related to the multimeric pattern.

VWF34

False negative and false positive responses in ristocetin-induced platelet aggregation mixing studies

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Background: Ristocetin-induced platelet aggregation mixing studies (RMS) are used to discriminate type 2B von Willebrand disease (2BVWD) from its phenocopy, Platelet-type von Willebrand disease (PT-VWD). Patient's platelet-poor-plasma (PPP) mixed with normal platelet-rich-plasma (PRP) stimulated with low dose ristocetin would show an enhanced aggregation in the presence of 2B VWD. PT-VWD would show negative responses in this RMS and instead cryoprecipitate challenge test may help its diagnosis.
Aims: To our knowledge, false positive and false negative results in RMS have never been reported. We therefore describe two pediatric patients with conflicting responses upon testing with RMS.
Methods: Results: Patient 1: A 10 year-old boy with mucocutaneous bleeding symptoms (ISTH-BAT:12), platelets: $210\times10^9L^{-1}$, VWF:Ag: 23%, VWF:RCo: < 5%, and enhanced response to low dose RIPA (0.5 mg/ml). RMS and cryoprecipitate tests were negative. Repeating RMS with other normal PRPs were also negative. However, exon 28 of VWF gene shows a p.Arg1308Lys mutation and a variant p.Ser1486Leu. His mother had the same phenotype, RMS and genetic findings. This indicates a false negative RMS.
Patient 2: A 14 year-old girl with primary immune thrombocytopenia ($6\times10^9L^{-1}$) unresponsive to IVIg and prednisone was evaluated for RMS to exclude 2BVWD associated thrombocytopenia.
After stimulation with ristocetin (0.5 mg/ml), a 70% of max aggregation was observed. Two weeks later, a new sample was drawn and RMS was repeated. Spontaneous aggregation (75%) was evident in RMS without the addition of ristocetin. No mutation was found on exon 28 VWF gene analysis. Patient′s PPP treated with Protein A Sepharose yielding IgG depleted plasma did not show spontaneous aggregation nor enhanced response to low dose ristocetin in a subsequent RMS study. This indicates a false positive RMS.
Conclusions: These cases are examples of variability in RMS, which should be considered for proper interpretation of such tests.

Women's Health Issues in Thrombosis and Hemostasis

WOM01

Phenotype of congenital Factor XIII deficiency in women: report of the French cohort

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Background: Congenital factor XIII (FXIII) deficiency is a rare bleeding disorder affecting around 30 patients in France. Besides its role in fibrin clot stabilization, FXIII is involved in placental attachment. Fetal miscarriages represent a frequent and concerning issue for these patients. Cumulated with the bleeding risk, pregnancies are especially at risk in this population.

Aims: The aim of our study was to describe gynecological and obstetrical events occurring in a French cohort of women with congenital FXIII deficiency.

Methods: We conducted a retrospective study in the French Bleeding Disorders Management Centers. Women between 15 and 65 year with a FXIII level < 10% were included. A case report form was fulfilled to gather events during their gynecological and obstetrical period.

Results: Eleven women were included. Deficiency in A-subunit was present in 7 patients, deficiency in A and B subunit in 2 and under investigations for 2 patients. Eight out of these 11 patients exhibited a FXIII level < 1%. Fetal miscarriage was the primary manifestation in 2 patients, the remaining were diagnosed during hemorrhage. With one exception, every patient received FXIII substitutionI. Menorrhagia were reported by 3 women, one patient suffered from menorrhagia despite substitution. Pregnancies (n = 13) were reported by 8 patients with 11 births, one on-going and one voluntary interruption of pregnancy. Every pregnancy was conducted under FXIII substitution, with a level target >10%. There was no consensual regimen. No hemorrhagic episode was reported. Four patients experienced at least one fetal miscarriage with a total amount of 30 miscarriages with 5 cases occurring during substitution. Except one patient who had hemorrhage and DIC during the management of miscarriage, none were transfused.

Conclusions: Altogether, our data confirmed the high incidence of miscarriage in these women, even under treatment. Pregnancies require substitution and we advocate for a standardized protocol in France.

WOM02

Abnormal platelet GPIbα and pregnancy outcome: an exploratory study

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Background: Pregnancy complications are known to increase in women with heritable platelet function disorders and there is evidence from literature that normal platelets may be important in placentation and pregnancy development. Fetal growth restriction was reported in association with defective platelet GPIbα in Bernard-Soulier syndrome but nothing was reported in association with the hyper-responsive protein causing PT-VWD.

Aims: To investigate whether hyper-responsive platelet GPIbα protein contributes to pathophysiological changes during pregnancy that lead to abnormal fetal outcome.

Methods: Pregnant females of transgenic mice with a C57/Bl6 background expressing normal (hTg ^{WT}) and hyper-responsive (hTg^{G233V}) GPIbα were sacrificed at gestational date (GD) 12.5 and 18.5. Placentae were removed and weighed. RNA was extracted from placentae from transgenic as well as normal C57/Bl6 mice and then reverse transcribed. PCR amplification of GPIbα was performed using human and mouse primers. The pups were removed, weighed and assessed for fetal growth restriction; defined as a fetal weight falling below the10th percentile of the hTg ^{WT} for that gestational age.

Results: PCR analysis showed that GPIbα is expressed in placenta of C57/Bl6 mice as well as the transgenic hTg ^{WT} and hTg^{G233V} animals. There was no significant difference in placental weight of hTg^{G233V} compared to hTg ^{WT} at GD 12.5 or 18.5. A significant reduction was found in fetal weights of hTg^{G233V} compared to hTg ^{WT} pups at GD 12.5 but not at GD18.5. However, no significant resorptions or fetal growth restriction were detected at GD 12.5 or 18.5.

Conclusions: GPIbα expression in the placenta may indicate its potential role in pregnancy but fetal outcome doesn't appear to be grossly impaired in association with a hyper-responsive platelet GPIbα. However, the abnormal fetal weight documented early in pregnancy calls for further analysis of placental structure and examination of placentation and vascular modeling before a final conclusion can be made.

WOM03

A meta-analysis of low-molecular-weight heparin to prevent pregnancy loss in women with inherited thrombophilia

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Background: Women with inherited thrombophilia have a modestly increased risk of recurrent pregnancy loss, however, whether the use of prophylactic-dose low-molecular-weight heparin (LMWH) during subsequent pregnancies improves this risk is unknown. The majority of trials evaluating LMWH in women with prior pregnancy loss include only a small proportion of women with thrombophilia.

Aims: In women with an inherited thrombophilia and prior late or recurrent early pregnancy loss, we sought to determine whether the use of LMWH reduced the risk of pregnancy loss when compared to no LMWH.

Methods: A literature search was conducted on MEDLINE, EMBASE and the Cochrane Database. Studies were included if they met the following eligibility criteria:

Table 1 (Abstract WOM03)

	Proportion with outcome in the LMWH group		Proportion with outcome in the No LMWH group					
	%	n/N	%	n/N	Relative Risk	95% Confidence Interval	P	I2 (%)
Primary Outcome								
Livebirth rate	84.5	201/238	65.0	159/245	0.81	0.55–1.19	0.28	91.9
Livebirth rate (multi-center trials)	83.5	132/158	82.4	136/165	1.04	0.93–1.16	0.52	12.9
Prior late loss (1 loss ≥ 10 wks)								
Livebirth rate	84.2	128/152	59.0	92/156	0.81	0.38–1.72	0.58	95.3
Livebirth rate (multi-center trials)	81.9	59/72	90.8	69/76	1.12	0.97–1.30	0.13	0.0
Prior early loss (≥ 2 losses < 10 wks)								
Livebirth rate (all multi-center trials)	86.5	32/37	86.2	25/29	0.97	0.80–1.19	0.79	N/A

(1) peer-reviewed randomized controlled trial,: (2) pregnant women with inherited thrombophilia and prior late (≥10 weeks) or recurrent early (≥2 losses < 10 weeks) pregnancy loss,
(3) randomly allocated to LMWH +/- aspirin (ASA), vs. no LMWH +/- ASA and (4) livebirth rate was reported.
Results: Eight trials and 483 patients met our inclusion criteria; investigators from 6 of the 8 trials provided additional data. There was no significant difference in livebirth rates with the use of LMWH compared to no LMWH (RR 0.81, 95% CI 0.55–1.19, P =0.28, I^2 91.9%). We performed a sensitivity analysis to explore multi-center vs. single-center trials as a cause of the high heterogeneity reported. When evaluating only multi-center trials there was no difference in livebirth rates between groups, with reduced heterogeneity (RR 1.04, 95% CI 0.93–1.16, P =0.52, I^2 12.9%). There was also no difference in livebirth rates with the use of LMWH vs. no LMWH when evaluating subgroups of women with prior late or recurrent early loss (Table 1).
Conclusions: LMWH (with or without ASA) did not reduce the risk of pregnancy loss in women with inherited thrombophilia with prior late or recurrent early pregnancy loss, when compared to no treatment or ASA alone.

WOM04

Prevention of recurrent pregnancy complications in patients with thrombophilia

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Background: Placental vascular complications remain the leading cause of maternal and perinatal morbidity and mortality.
Aims: Our objective was to evaluate maternal and fetal outcomes in women with history of pregnancy complications receiving the preconception treatment.
Methods: 500 pts with fetal loss syndrome, 160 pts with severe preeclampsia, 80 pts with venous thromboembolism (VTE), 100 ps with placental abruption, including 10 patients with multiple pregnancy after IVF, 100 pts with antenatal death and 500 healthy controls were tested to have genetic thrombophilia and antiphospholipid antibodies. Women with history of pregnancy complications received treatment in the preconception period and during pregnancy: low molecular weight heparin (LMWH) guided by D-dimer, natural progesterone up to 24–32 weeks, aspirin, antioxidants, vitamins of B group, folic acid (up to 4 mg in women with hyperhomocysteinemia).
Results: Thrombophilia was found in 75% pts with fetal loss syndrome and antenatal death, in 96% pts with recurrent preeclampsia, in 70% in pts with history of 1 episode of preeclampsia, in 80% pts with placental abruption and in 97,5% in pts with VTE. In the study group nobody had moderate or severe form of preeclampsia, mild preeclampsia was observed in 16%. All babies were alive. Preconception therapy allowed preventing recurrent fetal loss syndrome in 66%; 96% pts were delivered after 37 weeks. Patients had not recurrence of placental abruption or VTE.
Conclusions: Heterogenous thrombophilia might be the main pathogenetic mechanism of recurrent pregnancy complications. Due to thrombophilia involvement in trophoblast invasion and placentation early treatment is essential.

WOM05

Hematologic alterations in patients undergoing controlled ovarian stimulation for in vitro fertilization: a prospective observational pilot study

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Background: In vitro fertilization (IVF) is a unique model to study hematologic alterations, due to the rapid changes in endogenous estradiol (E_2) levels during controlled ovarian stimulation (COS). Supraphysiologic E_2 levels can result in venous thromboembolic events (VTEs) during or immediately after IVF. Such VTEs are most often due to concurrent ovarian hyperstimulation syndrome. The extent of alteration in the coagulation profiles of patients undergoing physiologic COS is currently unknown.
Aims: To investigate the hematologic alterations of patients undergoing COS for IVF.
Methods: Prospective, observational pilot study of patients < 40 years of age undergoing their first IVF cycle at our center. We recorded demographics, COS parameters, gonadotropins administered (IU), peak E_2 level (pg/mL), and number of oocytes retrieved. Hematologic parameters assessed on the day of COS start and hCG trigger included protein S activity, protein S free antigen, PTT, fibrinogen, von Willebrand factor (vWF), factor VIII, factor XIII, APC resistance and platelet function assay.
Results: Ten patients were enrolled, of which, 5 patients met inclusion criteria. The median age was 33 [range (31–35) years]. Patients underwent COS for 11 (10–12) days. The E_2 level of the day of hCG trigger was 2719 (1956.5–2995.5) pg/mL. The total number of oocytes retrieved was 19 (15.5–25). There was no difference in protein S activity (108 vs. 90%), protein S free antigen (128 vs. 100%), PTT (29.8 vs.

29.9 seconds), fibrinogen (259 vs. 225 mg/dL), factor VIII (115 vs. 126%), vWF antigen (111 vs. 120), factor XIII (120 vs. 100%), and platelet function assay (97 vs. 93 seconds) on the day of COS start and hCG trigger. APC resistance increased by 10.7% (6.5 vs. 5.6; P =0.03) from the day of COS start to hCG trigger. See Table.

Table Hematologic parameters.

Parameter	Day of IVF start	Day of hCG trigger	P
Protein S activity/ Protein S antigen free (%)	108 (92–121)/ 128 (103.5–128)	90 (78–97.5)/100 (83.5–106.5)	0.09/0.10
PTT (seconds)	29.8 (29.8–31.5)	29.9 (28.1–31.5)	0.92
Fibrinogen (mg/dL)	259 (212–286)	225 (212.5–268)	0.53
Factor VIII activity (%)	115 (101–130.5)	126 (121–155.5)	0.21
vWF antigen (%)	111 (83.5–135)	120 (97–168.5)	0.35
APC resistance	5.6 (5.05–5.85)	6.2 (5.80–6.55)	0.03
Platelet function assay (seconds)	97 (85.5–104.5)	93 (72–98)	0.35
Factor XIII activity (%)	120 (111.5–127)	100 (89–115.5)	0.08
Lupus anticoagulant (Silica clotting time)	1.1 (1.08–1.12)	1.09 (1.01–1.1)	0.25

Conclusions: Our preliminary findings do not indicate the presence of hypercoagulability during COS. A larger sample size is warranted to verify our findings.

WOM06

Sociodemographic characteristics of women with postpartum hemorrhage: a nationwide inpatient sample (NIS)-based analysis

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Background: Postpartum hemorrhage (PPH) is a major cause of maternal morbidity and mortality worldwide. While several clinical risk factors for PPH have been identified, less is known about the sociodemographic characteristics of women presenting with PPH.
Aims: We explored the racial, geographic, and hospital-based characteristics of women with PPH using a large national database, the National Inpatient Sample (NIS).
Methods: Retrospective analysis from the NIS database from 2009 to 2013. Inclusion criteria were female sex and discharge diagnosis of postpartum hemorrhage. Race, primary insurance payor, socioeconomic quartile, and hospital-based information (location, type, and bed size) were analyzed, as were hospital length of stay and in-hospital maternal mortality rates.
Results: 546,998 women had a recorded diagnosis of PPH during the study interval. Median age was 28 years (IQR 23–32). 49% of women were white, 24% Hispanic, 15% black, and 13% Asian or other. Primary insurance payor was Medicaid (46%), private insurance (48%), or self-pay/other (6%). 23% underwent Cesarean section. 42% of pregnancies were complicated by genital tract trauma and 11% by uterine atony. 16% of women received a red cell transfusion. 57% of women were managed at an urban teaching hospital, 32% in an urban nonteaching hospital, and 11% in a rural hospital. Median length of stay was 3.1 days and 285 women (0.1%) died in the hospital. Older age, black race, higher household income, larger hospital bed size and urban hospital location were significantly associated with longer hospital length of stay.
Conclusions: We used data from the NIS to explore the sociodemographic characteristics of over half a million women with pregnancies complicated by PPH. We identified several factors associated with longer hospital length of stay. Further in-depth analysis is planned, with the hope that PPH interventions can be targeted to areas of greatest need.

WOM07

The use of TEG/RoTEM in pregnancy and pregnancy associated complications: results of a worldwide survey by the ISTH SSC for women's health issues in thrombosis and haemostasis

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Background: Thromboelastography® (TEG®) and Thromboelastometry (ROTEM®) are viscoelastic tests that assess the kinetics of clot formation in the presence of cellular blood components. There is evidence from literature that these tests may not be used in full capacity to diagnose/monitor pregnancy associated coagulopathies.
Aims: To gather detailed information about the current worldwide use/applications of TEG/RoTEM in relation to pregnancy and its associated complications and to investigate the perceived challenges associated with their use.
Methods: An international online survey formed of 16 questions was distributed between Sep 2014 and Nov 2015, via emails to ISTH members, monthly ISTH newsletters, ISTH website and personal communications to a variety of gynecology and obstetric sites worldwide.
Results: 50 responses from 17 countries across the globe were received. 48% of participants had access to the analyzers (TEG:16%, RoTEM:24%, both:8%), mostly used for clinical (42%) vs. research purposes (27%) or both (31%) and accessed at a local laboratory or as bedside. 81% reported a use in the third trimester of pregnancy with a higher use in complicated (64%) vs. normal pregnancy. The tests were used mostly for postpartum hemorrhage, transfusion monitoring and DIC. Citrated native was the most frequently reported sample type (36%) with tissue factor-activated sample only reported by 14%. 73% followed the manufacturer's reference range with only 15% used in-house reference for normal pregnancy. Many challenges were reported including expenses, difficulty in interpreting results or making clinical decisions based on results, lack of test standardization and absence of reference range for normal pregnancy.
Conclusions: The current overall use of TEG/RoTEM is poor and is mostly limited to complicated third trimester pregnancy complications. Controlled trials showing clinical efficacy are required to enhance the confidence in the technology and enable wider applications.

WOM08

Hyperfibrinolysis as one of the reasons of recurrent fetal loss in the pregnant women

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Background: Fibrinolytic activation is a part of acute coagulopathy in pregnancy and may be the reason of fetal loss. Thrombelastometry (ROTEM) is able to measure the extent of fibrinolysis.
Aims: To evaluate the role of hyperfibrinolysis (HF) during 1st trimester (early gestation) in women with history of recurrent fetal loss due to haemorrhage and to investigate the diagnostic value of ROTEM to detect HF.
Methods: Fibrinolysis (F) was initiated in vitro by adding plasmin to kaolin-activated blood sample. Recorded parameter was TEG reaction time (r). The difference between r kaolin TEG and plasmin+kaolin TEG (rF) was calculated as percentage of rF prolongation time (normal range F= 70–130%). In control group there were 56 normal pregnant women. In investigated group - 139 pregnant women with one or more fetal loss due to haemorrhage.

Results: In control group we observed the tendency of inhibition of fibrinolysis (F = 92 ± 22%, range 61–77%). In the investigated group there were 27 women (19%) with HF (161±28%, p<0.05). In the last subgroup there were 11 women (41%) with bleeding disorders (F=243±22%, p<0.01). 7 patiens (63,4%) with HF and bleeding disorders were treated with tranexamic acid (TXA) during 5–7 days. TXA corrected rF to normal (F=103±18%) in a dose dependant manner. Normalization of rF was accompanied by cessation of bleeding.
Conclusions: HF may play significant role as a cause of bleeding disorders in the 1st trimester (early gestation). Thrombelastometry provides useful information on fibrinolytic activation. TXA applied in women with HF and bleeding allows to prevent hemorrhagic complications and improves perinatal outcomes.

WOM09

Risk assessment of venous thromboembolism (VTE) and thromboprophylaxis in women hospitalized with multiple pregnancy

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Background: Patients with multiple pregnancies are considered of high risk for developing venous thromboembolism (VTE) when hospitalized.
Aims: Evaluate the application of a thromboprophylaxis protocol with VTE risk score for multiple pregnancy women hospitalized in the Department of Obstetrics at Clinics Hospital University of Sao Paulo Medical School (HCFMUSP). Thromboprophylaxis employed in patients at risk for VTE. Assess the impact of application of this protocol in the prevention of maternal morbidity and mortality from VTE.
Methods: Longitudinal and prospective study of multiple pregnancies admitted in HCFMUSP in the period of December 2013 to November, 2015. Application of a thromboprophylaxis protocol with VTE risk score (see Table). Multiple pregnancy scored 1 in hospitalization. The collected data were analyzed descriptively, identifying the profile of multiple pregnancy women, using percentages, absolute values.

Table VTE risk score for hospitalized pregnant women.

SCORE 3	SCORE 2	SCORE 1
homozygous mutations	heterozygous FV Leiden/ heterozygous FII	parity≥ 3
combined thrombophilia risk factors	cancer (last 6 months)	multiple pregnancy
antiphospholipid syndrome	chemotherapy (last 6 m)	hyperemesis
cancer(stomach,lung, pancreas)	current severe infection	smoker≥ 20
inflammatory acute conditions (autoimmune diseases)	BMI ≥ 40 kg/m2	surgical procedure (except C-section)
sickle cell disease	Age ≥40y	gross varicose veins
nephrotic syndrome	lung disease (cyanosis)/postpartum hemorrhage >1 l	age≥ 35 and ≤ 39y
heart disease	immobility	
previous thromboembolism	Protein C/ S deficiency	

Results: We evaluated 145 twin pregnancies: 134 had one VTE risk score evaluation, 9 had two (multiple hospitalizations), 1 had 3 evaluations and 1 had 4 evaluations;42 clinical evaluations and 103 post-partum(22 vaginal deliveries and 81 c-section).Age range (15–46 y);38 cases were classified as high risk for VTE (26%) (score >3), 7 were already in anticoagulation (metallic cardiac valve, previous VTE). 31 patients received VTE prophylaxis with enoxaparin according to body weight and clinical conditions. One patient with score 5 developed EP due to a late introduction of prophylaxis (coagulopathy and hysterectomy). One patient with a low risk score had a DVT in the left leg in the 11o day after a c-section due to placental insufficiency of both fetuses. Score description in high risk score group: age 35–39 y(12 patients), parity ≥3(5),immobility(3), age >40y(9), serious infection(3), BMI > 40(10), gross varicose veins(1).
Conclusions: A quarter of women with multiple pregnancies were of high risk for VTE. The age and the high BMI were the main risk factors associated.

WOM10

Inherited thrombophilia influences localization and extent of pregnancy related deep venous thrombosis

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Background: Venous thromboembolism is one of the leading causes of maternal morbidity and mortality. The role of inherited thrombophilia in occurrence and localization of pregnancy related thrombosis is not clear.
Aims: To investigate the influence of hereditary thrombophilia on localization and extent of thrombosis in women with thromboembolic complications during pregnancy and puerperium.
Methods: We conducted a retrospective analysis of 212 consecutive women with pregnancy related deep vein thrombosis of lower extremities (LE) or pulmonary embolism (PE) who were referred to our institution for thrombophilia testing from January 2004 to December 2015. When DVT of LE was present with PE, the event was accounted as PE. Thrombosis of superficial veins was excluded. All thrombotic episodes were confirmed with duplex ultrasonography and CT pneumoangiography. In all women following causes of hereditary thrombophilia were tested: factor V Leiden and prothrombin G20210A mutations, antithrombin, protein C and protein S deficiency. Blood for thrombophilia testing was obtained at least 3 months after cessation of anticoagulant therapy.
Results: Out of 212 women with pregnancy related thrombosis, 33 (15,6%) developed PE, 73 (34,4%) developed unilateral thrombosis of both proximal and distal veins, 63 (29,7%) of proximal, and 36 (17%) of distal veins. Seven women (3,3%) developed concomitant bilateral thrombosis of deep veins of lower extremities. Hereditary thrombophilia testing was positive in 82/212 (38,7%) women. Prevalence of inherited thrombophilia was significantly higher in women with massive DVT (proximal+distal veins) and in women with isolated DVT of proximal veins than in women with PE and isolated DVT of distal veins.
Conclusions: According to our results, inherited thrombophilia influences localization and extent of pregnancy related thrombosis. Surprisingly, prevalence of thrombophilia was low in women who developed pregnancy related pulmonary embolism.

WOM12

Intravenous iron therapy in non-anemic iron-deficient menstruating adolescent females with fatigue

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Background: Menstruating women, particularly those with underlying bleeding disorders, are at increased risk for developing iron deficiency. Even in the absence of anemia, iron deficiency has been shown to adversely affect cognition and exercise performance. Orally

Table 1 (Abstract WOM12)

	Screening Mean (SD)	4th Infusion Mean (SD) [p value compared to screening]	6 Weeks Mean (SD) [p value compared to screening]	3 Months Mean (SD) [p value compared to screening]	6 Months Mean (SD) [p value compared to screening]
Ferritin (ng/ml)	13.40 (13.07)	224.30 (115.20) [P<0.0001]	141.50 (104.33) [P<0.0001]	81.68 (62.37) [P =0.0495]	85.20 (128.37) [P =0.0212]
Hemoglobin (g/dl) Anemic	11.32 (0.67)	11.58 (0.99) [P=0.9912]	12.67 (0.95) [P =0.0011]	12.84 (0.93) [P<0.0001]	12.96 (0.33) [P =0.0083]
Hemoglobin (g/dl) Non-Anemic	12.93 (0.66)	12.75 (0.88) [P =0.9999]	13.43 (0.46) [P =0.8532]	13.9 (0.63) [P =0.4634]	13.72 (0.67) [P =0.3002]
Iron (ug/dl)	53.20 (27.77)	83.67 (32.15) [P =0.0045]	80.98 (34.85) [P =0.0167]	87.18 (37.89) [P =0.0023]	88.89 (37.59) [P =0.0062]
Peds QL Patient	35.19 (16.79)	51.63 (21.55) [P =0.0035]	58.33 (21.29) [P<0.0001]	65.62 (17.90) [P<0.0001]	61.79 (19.46) [P =0.002]
Peds QL Parent	31.93 (19.61)	59.07 (23.65) [P<0.0001]	57.02 (24.40) [P<0.0001]	68.56 (21.74) [P<0.0001]	67.58 (26.12) [P =0.0004]

administered iron may be poorly absorbed and is often accompanied by side effects impacting patient complaince.

Aims: To demonstrate the effect of a standardized regimen of intravenous iron on the quality of life of a prospective cohort of young women with low serum ferritin and symptoms of fatigue.

Methods: Our study design was a prospective observational study. We included post-menarchal young women < 21 years of age with serum ferritin ≤ 20 ng/ ml and symptoms of fatigue by the Peds QL™ Multidimensional Fatigue Scale (Score ≤ 70) (Peds QL). Patients with hemoglobin < 10 g/dl were excluded. Patients received four infusions of intravenous iron sucrose over a 14-day period. Laboratory evaluations and the Peds QL, using child self-report and parent proxy-report were administered at last infusion, 6 weeks, 3 months and 6 month follow-ups. A linear mixed model was used to compare means over different time points.

Results: 22 young women (ages 14–20 years) were recruited. There were 15 patients with heavy menstrual bleeding, 7 with platelet function defects, 1 with type-1 von Willebrand disease and 1 with Ehlers Danlos syndrome. Infusions were well tolerated except for four patients with headache and nausea. Results for ferritin, hemoglobin, iron and the PedsQL scores are summarized in Table 1. These results demonstrate efficacy as well as sustained responses.

Conclusions: Our results demonstrate that intravenous iron objectively improves fatigue and quality of life in young women with iron deficiency and mild/no anemia. In non-anemic patients, iron administration did not influence the hemoglobin concentration. Thus the fatigue-reducing effects of iron therapy reflect the non-hematological functions of iron.

WOM13

Thromboelastography in pregnancy: establishing reference ranges in third trimester and exploring predictive value for postpartum haemorrhage

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Aims: To establish reference ranges for thromboelastography (TEG) in term pregnancy and explore its role in predicting postpartum haemorrhage (PPH).

Methods: 101 women at ³36 weeks gestation consented to a prepartum blood test for TEG analysis and review of their medical notes postpartum. Four women were excluded from the primary aim of determining TEG reference ranges due to obstetric conditions which might affect clotting.

Participants had a mean age of 33.1 years (range 21–45), gestation at time of sampling 39.1 weeks (36–41) and parity nulliparous (36.3%), primiparous (36.3%) or multiparous (26.7%).

Results: The association between prepartum TEG parameters and estimated blood loss (EBL) was investigated using the two-tailed Spearman test. This found a statistically significant negative correlation between *MA* (maximum amplitude, ie clot strength) and EBL (P =0.023*)*. By extension, *G*, the log-derivative of *MA*, was also statistically significant. The association between *MA* and EBL was further supported by Mann-Whitney-U analysis (p = 0.0139) and Kruskall-Wallis test (P =0.0181). Clinical incidence of PPH was compared in subgroups of women with low, intermediate or high values of *MA* and found a borderline significant correlation (p = 0.0604, Fisher's Exact Test). After adjusting for possible confounding factors (age, parity and mode of delivery), the significance remained (P = 0.0532).

Conclusions: This study has added to limited existing data on reference ranges for TEG at term pregnancy. It has also for the first time identified a correlation between *MA* and postpartum haemorrhage. Further research is required into the mechanisms by which this occurs and potential clinical implications.

WOM14

Screening of female family members of von willebrand disease patients: utility of a screening tool

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Background: Reproductive age female family members of patients with Von Willebrand disease (VWD) may be at increased risk for bleeding and yet frequently remain undiagnosed and outside the care of hematologists.

Aims: The purpose of the study was to evaluate the utility of a modified Screening Tool in a racially diverse population of reproductive age female family members of VWD patients.

Methods: 81 women including 37 reproductive age female family members of VWD patients, 19 previously diagnosed female VWD patients and 25 healthy female controls were administered a modified Screening Tool (Philipp et al, Development of a Screening Tool for Identifying Women with Menorrhagia for Hemostatic Evaluation. Am J Obstet Gynecol 2008; 198(2):163.e1-8) and had blood drawn for CBC, ferritin, and VWF testing. Participants completed a pictorial blood assessment chart (PBAC) at their next menses.

Results: Low VWF (≤50%) at the time of study was found in 16% of family members, 47% VWD patients, and 4% controls (P =0.001). Low ferritin (< 30 ng/ml) was found in 50% of female family members, 33% VWD patients, and 28% controls (P =0.19). Hgb< 12 g/dL was found in 26% of family members, 24% VWD patients, and 8% controls (P = 0.20). The screening tool was positive in 32% female family members, 79% VWD patients, and 16% controls (P< 0.001). In family members, incorporating low ferritin with the screening tool resulted in a sensitivity of 83% (95% CI, 36–100), NPV 93% (95% CI, 68-100) with 17% false negatives.

Conclusions: These data suggest that a modified Screening Tool in combination with low ferritin may help stratify female family members of VWD patients who may benefit from hemostatic testing and referral.

WOM15

Coagulation factor changes during pregnancy in women with inherited bleeding disorders

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Background: In normal pregnancy there is an overall change in coagulation towards thrombosis. This is reflected by the increase in most coagulation factors seen during pregnancy. The degree of change for women with inherited bleeding disorders (IBDs) is still not fully understood. Often women with IBDs will only correct their factor level to a normal non-pregnant level. With haemorrhage still the leading direct cause of maternal death and women with IBDs at an increased risk of this complication it is important to improve knowledge in this area.
Aims: To publish average factor levels pre-pregnancy and during each trimester of pregnancy for women affected by von Willebrand disease (VWD), FXI deficiency, FVII deficiency and carriers of haemophilia A and B.
Methods: We studied women with IBDs attending multidisciplinary haematology/obstetric clinics between 2001 and 2011 at the Royal Free Hospital, London UK. Data from 248 pregnancies in 188 women were included. The study had ethical approval and written informed consent was obtained from participants. The data were analysed to compare pre-pregnancy levels with levels during each trimester of pregnancy. The study is ongoing and we will present an update to include data from pregnancies from 2011–2015.
Results: Compared to pre-pregnancy, statistically significant increases in factor levels were found for each trimester in women with VWD and carriers of haemophilia A. A significant rise in FIX was seen in the third trimester for carriers of haemophilia B. Further analysis will be conducted with final data collection to assess changes in FXI and FVII.
Conclusions: We found that mean factor levels in the third trimester were below proposed reference ranges for pregnant women for all conditions studied apart from VWD (FVIII level). This is supportive of recent suggestions that the current practice of basing management decisions on non-pregnant parameters may not sufficiently mimic normal physiological processes.

WOM16

Effect of pregnancy on the course of immune thrombocytopenia: a retrospective multicenter study

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Background: Retrospective data show that severe immune thrombocytopenia (ITP) during pregnancy is relatively uncommon, being in the range of 15%.
Aims: In this multicenter study, we evaluated the course of ITP during pregnancy and defined maternal risk factors predictive of ITP flares.
Methods: Data was retrospectively obtained from pregnant women with ITP who were admitted to various hematology centers in Turkey. Demographic and clinical data were obtained from medical charts. The study was approved by the local ethical committee.
Results: 74 pregnant women with ITP were included (median age:30, range:17–46). 47 were diagnosed with ITP during pregnancy; others had been diagnosed before pregnancy. 25 of the patients had used any treatment for ITP before pregnancy. 9 patients had used steroids; 8 patients had used steroids and/or IVIG therapy. Splenectomy was performed in 8 patients before pregnancy after steroid failure. The median platelet count nadir during pregnancy was 43000/mm^3 (1 1000–113 000). In 22 pregnancies (29.7%), platelet count decreased to < 30000/mm^3 at least once during pregnancy. Mucosal and/or cutaneous minor bleeding developed in 5 pregnant ITP patients; there was spontaneous abortion in 3 patients; and one baby had intrauterine growth retardation.During pregnancy, 19 patients were given steroids because of thrombocytopenia, 6 were treated with IVIG, and 13 were administered both steroids and IVIG. The median platelet count at the time of delivery was 87000/mm^3 (30000–256000). The type of delivery was vaginal in 48.5% of the pregnancies. Postpartum bleeding occurred in 3 patients. 4 ITP patients were given erythrocyte suspensions.There were no maternal deaths. Only 2 newborns needed specific therapy for neonatal thrombocytopenia.
Conclusions: In our series, most patients were diagnosed with ITP during pregnancy (63.5%). There were no major complications, other than one spontaneous abortion. Treatment indication was present in only a few newborns.

WOM17

The clinical presentations and management of hermansky-pudlak syndrome in the obstetric and gynaecology setting - a systemic review

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Background: Hermansky-Pudlak syndrome (HPS) is one of the rare autosomal recessive syndromes characterised by storage pool deficient platelets, lysosomal ceroid lipofuscin deposits and tyrosinase positive oculocutaneous albinism. The clinical manifestations include bleeding diathesis, multi-organ disease (such as pulmonary fibrosis, granulomatous colitis) and degrees of oculocutaneous albinism thus can present in different medical specialities. In the Obstetric and gynaecological (O+G) setting, it can be associated with significant life-threatening bleeding sometimes necessitating high units of blood transfusion. Pulmonary fibrosis, granulomatous colitis and renal failure all pose significant medical and anaesthetic risks in these patients.
Aims: Most of our clinical knowledge is from case reports. This systemic review collates the available case reports in O+G, its presentations and management strategies.

Methods: 11 pregnancies in 7 women and 2 gynae patients were identified after extensive literature research using MEDLINE, EMBASE, COCHRANE and PUBMED.
Results: The diagnostic age ranged from 6 to 42 years and common in Puerto Ricans. Most cases presented with easy bruising, menorrhagia, epistaxis, visual impairment. 2 cases were diagnosed after a massive obstetric haemorrhage (MOH). In 9 obstetric cases with known HPS, 4 had prophylactic DDAVP out of which 3 needed platelets and red cells transfusions to treat MOH; 4 had prophylactic platelets transfusion with no MOH or further treatment; and 1 had no prophylaxis with normal outcome. All delivered at term - 6 normal deliveries and 5 caesarean sections for obstetric reasons. Regional anaesthesia was avoided. The 2 gynae cases presented with severe menorrhagia and needed packed red cells transfusion, hormonal treatment with norethisterone or OCP, tranexamic acid, DDAVP or rFVIIa.
Conclusions: MOH or menorrhagia in a patient with OCA, think 'HPS' and involve senior colleagues including the haematologist early to start the right treatment. This can save their life!

WOM18

Proposed trial: HYPATIA - a prospective randomised controlled trial of hydroxychoroquine vs. placebo during pregnancy in women with antiphospholipid antibodies

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Background: Antiphospholipid syndrome (APS) is defined by the presence of antiphospholipid antibodies (aPL) with thrombosis and/or obstetric morbidity. The obstetric morbidity includes recurrent first and second trimester loss, stillbirth, intrauterine death, pre-eclampsia, premature birth and fetal growth restriction (FGR).
Current treatment including low dose aspirin and low molecular weight heparin have improved pregnancy outcome, but reduction in pregnancy loss remains sub-optimal.
Hydroxychloroquine (HCQ) has been used for decades in patients with SLE to prevent flares and has retrosapective data suggests it may have an antithrombotic effect in aPL positive SLE patients. Our retrospective study suggests that HCQ has a favourable effect on pregnancy outcomes in aPL positive patients. As HCQ is recognised to be safe in pregnancy, we propose a RCT of HCQ vs. placebo in pregnant women with aPL.
Aims: To undertake a randomised controlled trial of HCQ vs. placebo in addition to standard treatment in women with aPL in order to improve pregnancy outcome.
Methods: We will conduct the first multicentre RCT of HCQ vs. placebo in addition to standard treatment in pregnant women with isolated aPL or APS. A total of 328 women will be enrolled.
Results: The primary outcome will be a composite of adverse aPL-related pregnancy outcomes, including: miscarriage < 10 weeks' gestation, miscarriage >10 weeks' gestation and premature birth due to PE < 34 weeks, eclampsia or FGR. Secondary endpoints include: gestational age at delivery, birth weight, mode of delivery, APGAR score, neonatal morbidity (bleeding or thrombotic complications, infections, congenital abnormalities), days to hospital discharge following delivery (mother & child) and thrombotic events during pregnancy and 6 weeks post partum.
Conclusions: The trial will address the issue of the utility of HCQ in improving pregnancy outcome in women with aPL. We invite other centres to express interest in joining us.

WOM19

First venous thromboembolic episodes in women with antithrombin deficiency often associated with oral contraceptives or pregnancy

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Background: Patients with antithrombin (AT) deficiency have an estimated 20–50% lifetime risk of venous thromboembolism (VTE). Women with AT deficiency may be at additional risk for VTE in the setting of hormonal exposure, but this risk has not been well quantified.
Aims: To examine the relationship between hormonal stimulation (estrogen-containing oral contraceptives and pregnancy) and risk of VTE in women with AT deficiency.
Methods: Retrospective review of female patients selected from a Mayo Clinic congenital AT deficiency database. Medical records were reviewed for evidence of VTE and hormonal exposure at the time of VTE. Conventional statistical methods were utilized.
Results: 17/23 women with AT deficiency experienced ≥1 VTE events. Index VTE events occurred at a median age of 24 years (range 16 to 54). 4/17 (24%) of index VTE events occurred during pregnancy, and 7 of 17 (41%) occurred with OCP use. Only one of these women had a known diagnosis of AT deficiency at the time of the index VTE. Women who experienced first VTE with hormonal stimuli were diagnosed with AT deficiency at a median age of 28 years, while those who experienced first VTE in the absence of hormonal stimuli were diagnosed at a median age of 45 years. The diagnosis of AT deficiency in women without history of VTE was based on testing in the setting of known family history of AT deficiency.
Conclusions: The majority of women with AT deficiency (65%) developed VTE in the setting of exposure to hormones, and hormonal stimulation is a powerful risk factor for thrombotic events in these women. Investigation into the role of genotype as a predictor of VTE is ongoing. Further evaluation of the impact of AT deficiency on women's decision-making regarding contraception and pregnancy is warranted.

WOM20

Evaluation of protein c and protein s levels in preeclamptic women seen in a tertiary institution in the niger-delta region of nigeria - any role in the development of preeclampsia?

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Background: Preeclampsia contributes significantly to maternal, fetal and neonatal morbidity and mortality, however, its cause is poorly understood. The role of thrombophilia in the pathogenesis of preeclampsia is controversial.

Aims: To evaluate the levels of protein C and protein S in women with preeclampsia and to determine if there is any association between their levels and development of preeclampsia.
Methods: This was a hospital based case-control study. Protein C and protein S levels were evaluated in citrated plasma of 90 women whose pregnancies were complicated by Preeclampsia (cases) and 90 women who had normal pregnancy (controls). Protein C and free protein S antigen levels were analyzed using ELISA-based methods, while protein C activity level was assessed using a chromogenic
(Protac end point) method.
Results: The mean levels of protein C antigen and activity did not differ significantly in Preeclampsia and normal pregnancies (p-values = 0.639 and 0.444 respectively). The frequencies of protein C antigen and activity deficiency were found to be similar in preeclampsia and in controls (36.7% vs. 44.4%, p-value 0.288; and 6% vs. 6%,p-value 1.000 respectively). The mean level of free protein S antigen was higher in preeclampsia than in normal pregnancies (p-value = 0.004). None of the controls had protein S deficiency while 2 (2.2%) of the women with preeclampsia were protein S deficient; this was however not statistically significant (p-value 0.497). Therefore there seem to be no significant association between these proteins (protein C antigen and activity; and free protein S) and preeclampsia.
Conclusions: Disorders of protein C and free protein S are unlikely to be associated with preeclampsia and thus may not have a role to play in its aetiopathogenesis. Therefore if these results are validated by other more broad-based studies in our environment then, routine assessment of these natural anticoagulants in women with preeclampsia will not be justified.

WOM22

High Factor VIII and advanced disease are associated with risk of recurrent thrombosis in women with breast cancer

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Background: A recently proposed predictive model for risk assessment of venous thromboembolism (VTE) in cancer patients included determination of FVIII activity as a novel biomarker.
Aims: Our study aimed to investigate the association of FVIII with the risk for recurrent VTE during breast cancer treatment.
Methods: Total 100 women who developed thrombosis during the breast cancer treatment were included in the study and followed up with for 36 months. The final end point that was observed in the study group was recurrent venous thrombosis (RVT). At the same time 100 women with breast cancer without VTE as a control group, were included in order to define the median and IQR of FVIII in breast cancer patients.
Results: RVT was observed among 17% of the study participants. Patients that developed RVT had significantly higher FVIII; median (IQR), of 1.94 (1.17) than those who stayed free of recurrent during follow-up; 1.61 (0.71); P =0.045. In RVT group, 59% of women had FVIII above the 75th percentile of controls. Recurrent thrombosis occurred in 9% of VTE women who were considered to be in an early stage of breast cancer and in 31% of women who were considered to be in the advanced stage of the disease, P =0.002. High FVIII and advanced disease are associated with risk for recurrent thrombosis with an OR of 6.23 (95% CI 1.042–37.289), respectively OR 10.43 (CI 2.305–47.264).
Conclusions: Our study results showed that FVIII as a marker of hypercoagulable state represents a contributing factor for the recurrence of VTE in women with breast cancer.

WOM24

Multicenter registry on thrombophilia and pregnancy loss: the OTTILIA study

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Background: Pregnancy loss (PL) affects up to 10–15% pregnancies/year (with 1% of recurrent events, RPL). Acquired and inherited thrombophilias have been associated with RPL and a beneficial effect of antithrombotic prophylaxis has been hypothesized. Available findings, however, are conflicting.
Aims: To better define the management of pregnancies in women with otherwise unexplained spontaneous and recurrent PL with/without thrombophilias.
Methods: Consecutive pregnant women undergone thrombophilia screening and with a history of RPL ($\geq$ 3 or 2 in the presence of at least 1 normal fetal karyotype) or at least 1 intrauterine fetal death (IUFD, i.e. a loss after 20 weeks of a normal fetus) are eligible for the registry (clinical Trials.gov, NCT02385461). All involved Centres are asked to include information on pregnancy, prophylactic approaches and outcomes into a dedicated database and to follow up women until delivery.
Results: As of February 1st 2012, 140 women with RPL (n= 90) or IUFD (n= 50) have been enrolled at 9 Centres. Median age is 35 years (range 21–47); 48 (34.3%) carry Factor V Leiden (n= 25) or prothrombin 20210A mutation (n= 23), respectively; 10 (7.1%) carry severe thrombophilias (1 both the mutations, 4 antiphospholipid antibodies, 4 protein S and 1 protein C deficiency). 164 pregnancies were started

Table 1 Management of pregnancies in different groups.

Index-pregnancies (n= 164)	No prophylaxis	ASA	LMWH	ASA+LMWH
Pregnancies in FVL heterozygotes, n (%) (n= 28)	1 (3.6)	2 (7.1)	19 (67.9)	6 (21.4)
Pregnancies in PTm heterozygotes, n (%) (n= 26)	1 (3.8)	0	22 (84.6)	3 (11.6)
Pregnancies in carriers of severe thrombophilias, n (%) (n= 11)	0	0	5 (45.4)	6 (54.6)
Pregnancies in non carriers, n (%) (n= 99)	37 (37.3)	17 (17.2)	28 (28.3)	17 (17.2)
FVL: factor V Leiden	PTm: prothrombin 20210A mutation	ASA: low-dose aspirin	LMWH:low-molecular-weight heparin	

and 21 women became pregnant more than once. Treatment with antithrombotic drugs (low-dose aspirin, prophylactic doses of low-molecular weight heparins) was used in 104 (63.4%) pregnancies (Table 1). Outcome is so far available in 145 pregnancies; 3 were lost to follow-up, 16 are ongoing.

Conclusions: In women with previous PL, antithrombotic prophylaxis is mostly prescribed in pregnant women carrying thrombophilia, whereas about 40% on non-thrombophilic women do not receive any antithrombotic treatment. A larger sample size is required to compare outcomes in different groups of women and to define strategies to reduce the risk of recurrence.

WOM25

Management of pregnancy in vWD patients

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Background: vWD patients with various types and severity of the disease were followed-up and treated in our centre in order to prevent bleeding complications during pregnancy, delivery and early postpartum period.

Aims: To present single centre experience in management of pregnancy in patients with vWD.

Methods: Twelve pregnancies in nine vWD patients were managed in our centre between 2008 and 2015. 2 patients (3 pregnancies) had vWD type 1, 6 patients (8 pregnancies) had type 2A, 1 patient had type 2N. The patients were checked up every trimester and vWF and FVIII levels were measured. Prophylaxis with FVIII/vWF concentrate was administered to patients with bleeding complications of pregnancy or in order to prevent excessive bleeding loss during labor and early postpartum period in patients with incomplete rise in vWF/FVIII levels. The doses of peripartum prophylaxis varied between 8–50 IU/kg. Most of the patients received peripartum anticoagulation prophylaxis with LMWH.

Results: All the pregnancies were succesful. A prompt increase in FVIII and vWF to normal levels was observed in patients with type 1 vWD, whereas the rise in level of factors was incomplete in all patients with type 2 vWD. However, all these patients except of one had no bleeding complications during their pregnancies. Primary postpartum hemorrhage despite prophylaxis with FVIII/vWF concentrate complicated only two of the twelve deliveries.

Conclusions: Pregnancy in patients with vWD should be managed by a multidisciplinary team including hematologist, obstetrician and anesthesiologist. Regular monitoring of vWF/FVIII levels during pregnancy is necessary. Prophylactic treatment with FVIII/vWF concentrate has to be given as treatment of bleeding complications as well as prevention of excessive bleeding during delivery in case of incomplete rise in vWF/FVIII level. Anticoagulation prophylaxis in early postpartum period is safe and does not increase bleeding risk in patients with vWD.

WOM26

The dependence of low molecular weight heparin efficacy (Tested by Anti Xa) on the level of antithrombin - a case report of a pregnant patient with antithrombin defficiency

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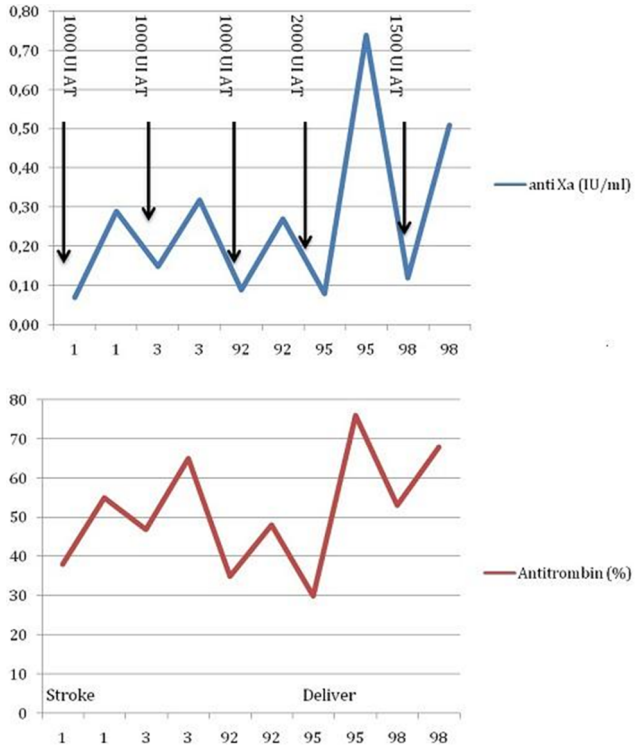
Background: 24-year old patient was first examined at our department in her 8th week of the 3rd pregnancy (previous pregnancies lead to spontaneous abortion). She was treated for hypertension and had ischemic stroke 2 years ago, at that time she was diagnosed with antithrombin defficiency with homozygote causal mutation c.2759c-t. Serum level of antithrombin varied between 20–30%. The patient was treated with LMWH in prophylactic dose (higher dose was not possible due to gynaecological haemorhage) together with ASA. With the beginning of the second trimester she underwent recurrent stroke with expression aphassia and right-sided hemiparesis with the finding of ischemia of left ACM (based on MRI). Trombolytic therapy was not indicated due to the patient's disaapproval. We increased LMWH to therapeutic dosing followed by prompt recovery of motoric functions with persistence of slight expression aphasia. The patient then changed LMWH for warfarin and was stable until the 29th week of pregnancy when she developed preeclampsia. Due to the need for premature termination of her pregnancy she translated back to therapeutic dose of LMWH tohgether with substitution of antithrombin. Following adequate anti-hypertension and symptomatic treatment her condition stabilized, and the obstetricians decided to postpone the delivery due to the immaturity of the fetus. In the 30th week of pregnancy the fetus has demised. After the stillbirth, we re-started warfarin therapy which lasts up to now. The patient is without any neurological defficiency.

Methods: Case report

Results: Graph 1 shows the dependence of antiXa levels on the level of antithrombin (the measurements were acquired before and after antithrombin substitution with identical interval after LMWH).



Graph 1

Conclusions: We demonstrate generally known fact about antithrombin bening the co-factor of low molecular weight heparins, and the importance of antithrombin substitution in the treatment of thrombotic complications in the case of its defficiency. Grant LF 2016-001

WOM27

Yes or no for anticoagulant thromboprophylaxis in pregnant women with repeated pregnancy loss?

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Background: Thrombophilic state increases the risk of placenta-related pregnancy complications, which may be prevented by low-molecular-weight heparin (LMWH). According to the results of the recent meta-analysis, in patients with/without thrombophilic state, LMWH provided the highest probability of live childbirth and thus, it should be given. Currently, the administration of LMWH in patients with inherited thrombophilic state and repeated pregnancy loss is not recommended. Only pregnant women-homozygous for factor V Leiden or prothrombin mutation and family history for venous thromboembolism (VTE) should receive ante- and postpartum thromboprophylaxis. However, recommendations are mainly made according to case series, derivations from non-pregnant patients and opinion of specialists.
Aims: To evaluate the effectiveness of ante- and postpartum anticoagulant prophylaxis in the prevention of recurrent pregnancy loss.
Methods: From February 2014 to December 2015, the effectiveness of prophylactic dose of LMWH in 26 women with thrombophilia and repeated pregnancy loss ($n \geq 2$) was evaluated from the first trimester of pregnancy until the end of puerperium. Changes in hemostasis, occurrence of bleeding, VTE, pregnancy and other complications were monitored.
Results: In 26 patients with recurrent pregnancy loss and thrombophilia, LMWH once daily was administered. Monitoring of hemostatic parameters was performed at 10–12, 16–18, 26–28, 35–36 gestational weeks and 6–8 weeks after delivery. Dose of LMWH was titrated to achieve a sufficient anticoagulant level with respect to the changes in hemostasis and body weight. In all women, pregnancy was successfully ended by delivery at term without serious complications.
Conclusions: Anticoagulant thromboprophylaxis in women with thrombophilia and 2 or more pregnancy losses improves pregnancy outcome and monitoring of hemostasis in high-risk pregnancy seems to be useful. However, randomised prospective controlled trials are required to confirm our conclusions.
We thank Vega 1/0168/16

WOM28

The role of LMWH to improve the pregnancy outcome

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Background: Heparin not only acts as an anticoagulant but also have immunemodulatory and anti-inflammatory effect. Heparin alter the hemostatic response to controlled ovarian stimulation and modify the risk of thrombosis. It can also modulate many of the fundamental physiological processes required for blastocyst apposition, adherence and implantation and as well as trophoblast differentiation and invasion due to its similarities with heparan sulphates and has the potential to improve pregnancy rates and outcomes. However, the role of LMWH to improve the pregnancy outcome is still controversial.
Aims: Evaluate the effect of heparin on the pregnancy outcome.
Methods: A retrospective observational analysis of 962 women with thrombophilia (inherited thrombophilia including factor V Leiden, prothrombin mutation, MTHFR mutation, plasminogen activator inhibitor-1 (PAI-1) mutation, protein S and C deficiency, antithrombin deficiency) and a history of unsuccessful reproduction (total 1104 pregnancies - spontaneous or after IVF) we evaluated the effect of LMWH on pregnancy outcome.
Results: The treated group: significantly more births, the risk of miscarriage reduced 5-fold. No statistical difference between the presence of inherited thrombophilia and pregnancy rate in treated and untreated.
Conclusions: Our results found a significant higher pregnancy rate in women with previous recurrent pregnancy loss was observed with LMWH and no relation among inherited thrombophilia and pregnancy rate.

WOM29

Antibodies to Factor XII and heterozygous factor V Leiden thrombophilia: are they responsible of intrauterine fetal death in a case of varicella infection during pregnancy?

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Background: Factor XII (FXII) is a component of the contact system of the blood coagulation. It's required for a normal activated partial thromboplastin time (aPTT) but it doesn't appear to be required for normal coagulation. FXII deficient patients may experience thromboembolic events. Otherwise, antibodies to FXII (FXIIabs) are less reported than FVIIIabs or FIXabs and showed curiously a strong association with fetal loss.
Aims: In this report, we aimed to explain why a 20-year-old asymptomatic woman showed a severely prolonged aPTT with normal prothrombin time (PT) at the time of labor induction at 36 weeks amenorrhea because of intrauterine fetal death following maternal varicella infection.
Methods: Platelet poor plasma was prepared by a double centrifugation of the citrated whole blood. PT, aPTT, thrombin time (TT) and factors clotting activity were measured using the STA Compact (Diagnostica Stago, Asniers, France). The correction of aPTT after 1:1 mixing with normal plasma was assessed before and after 2 h of incubation at 37°C and then the Rosner index was calculated (RI). The screening of inherited thrombophilia was also investigated.
Results: Laboratory tests at the time of labor induction were as follows: PT was 90%, aPTT was 81s/30s, TT was 18s/19s and fibrinogen was 3,5 g/l. The prolongation of aPTT was checked on another sample. Mixing the plasma with an equal volume of normal plasma corrected the patient's prolonged aPTT (39s, RI of 10%). Clotting factor levels of the intrinsic system were as follow: FVIII: 120%, FIX: 90%, FXI: 81% and FXII: 1.5%. FXIIabs were so suspected. aPTT was no longer corrected with normal plasma after 2 h of incubation at 37°C (54s, RI of 29%). FXIIabs was confirmed by Bethesda method. Surprisingly, the patient was found heterozygous for the factor V Leiden mutation.
Conclusions: FXIIabs and FV Leiden thrombophilia may have synergistic amplification of placenta-mediated pregnancy complications.

Working Group on Perioperative Thrombosis and Hemostasis

WOR01

Absence of pre-operative VWF workup is associated with increased intraoperative bleeding during Wilm's tumor resection

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Background: Acquired von Willebrand Syndrome(aVWS) is a rare condition associated with Wilm's tumor(WT) which may not be managed adequately by standard massive transfusion protocol. Therefore, pre-operative assessment of von Willebrand Factor(VWF) can help to avoid potential bleeding complications.

Aims: The aim for this study is to assess the relationship between aVWS and surgical estimated blood loss(EBL) among pediatric patients who underwent WT resection.

Methods: Medical records of patients who underwent WT resection between 2008 to 2014were retrospectively reviewed to collect pre-op coagulation workup results when available including PT, PTT, platelet counts, VWF workup and EBL recorded in the operative notes. aVWS was considered present when VWF:Act/Ag < 0.7. Non-parametric statistical analysis was performed and a p value of < 0.05 was considered significant.

Results: Of 65 patients [age 3(2–5), median (IQR)] in this study only 18 (27.7%) were evaluated for aVWS, and of these 18 patients, 9 patients (13%) met criteria for aVWS, the remaining 9 were negative for von Willebrand Disease. In patients with aVWS, pre-op plans were implemented to address increased bleeding risk including treatment with HUMATE-P. Thirty-five patients (54%) had recorded EBL and there was trend toward significance (P =0.12) for more severe intraoperative bleeding amongst those without pre-op VWF workup vs. those with VWF workup (normal & aVWS). Specfically. 2 untested patients were outliers with the highest EBL at 43.84 and 227.27 mL/kg respectively; neither patients had prior bleeding history or symptoms. There was no significant difference (p>>0.05) in PT, PTT, platelet counts and tumor size.

Conclusions: Our study demonstrated that routine pre-op coagulation workup for WT resection with PT, PTT and platelet count alone may be inadequate. Pre-operative VWF assessment identified laboratory criteria for aVWS in 13% of patients and allowed for formulation of a treatment plan to address severe potential intraoperative bleeding.

Table EBL among Unknown vs. Normal vs. aVWS. (Abstract WOR01)

n=35		EBL (mL/kg)	
VWF Status	Median	Percentile 25	Percentile 75
Unknown	6.50	1.62	14.55
Normal	6.62	2.01	9.68
aVWS	2.91	1.73	4.36

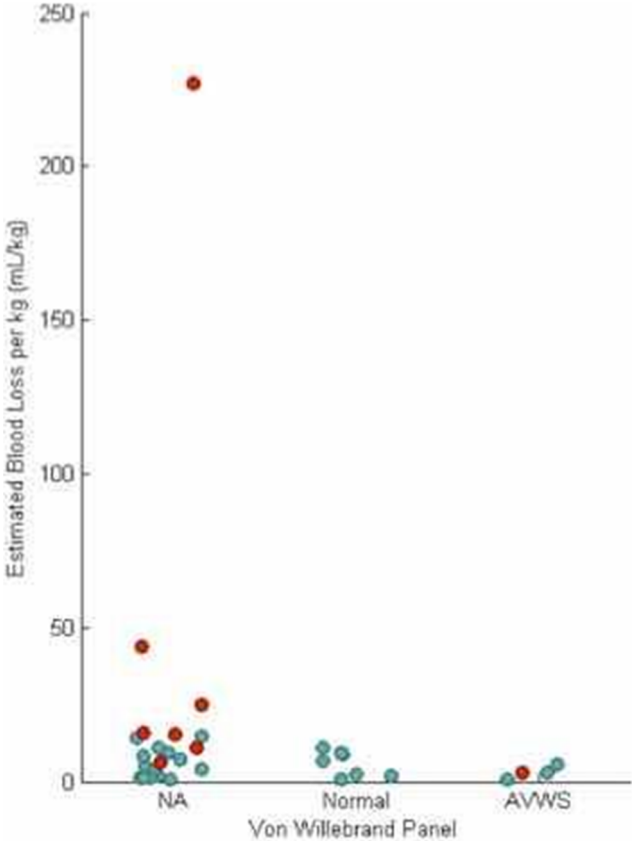

Figure EBL among Unknown (NA) vs. Normal vs. aVWS. (Abstract WOR01)

WOR02

High variation of peri-operative bridging use in real-world practice: results from a clinician survey

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Background: In June 2015, the BRIDGE study demonstrated that peri-operative LMWH bridging anticoagulation causes harm without benefit for patients with atrial fibrillation (AF).

Aims: To assess how the BRIDGE study has affected how physicians approach peri-operative bridging anticoagulation.

Methods: An electronic survey was administered to all primary care providers, cardiologists and gastroenterologists at the University of Michigan in August 2015 (IRB approved). Participants were presented with four hypothetical scenarios of AF patients who must interrupt warfarin for a colonoscopy. The patients had varying numbers of stroke risk factors (hypertension, diabetes, heart failure and prior stroke). Providers were asked if they would recommend bridging anticoagulation for each scenario. We used multivariable logistic regression to calculate rates of bridging anticoagulation adjusted for the interaction between the estimated stroke risk (by $CHADS_2$ score) and prior stroke as well as clinician-specific factors (including specialty).

Results: Surveys were completed by 134/262 (51.1%) of invited clinicians. Adjusted rates of bridging anticoagulation increased as the number of stroke risk factors increased, from 6% in the lowest stroke-risk scenario to 87% in the highest stroke-risk scenario (see Figure). A prior stroke was a stronger predictor of bridging use than the

estimated stroke risk ($P< 0.001$). Provider specialty strongly influenced the decision to bridge (see Figure). For example, in a $CHADS_2$=3 patient without prior stroke, cardiologists were least likely to recommend bridging (17.4%) while family medicine (62.5%) and gastroenterologists (40%) were more likely to recommend bridging ($P< 0.001$).

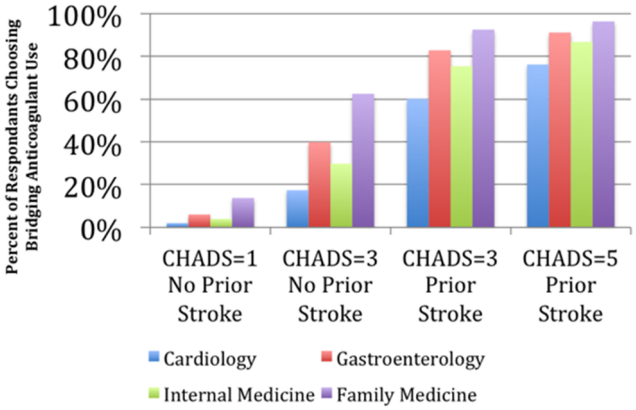

Figure Bridging Use by Stroke Risk.

Conclusions: Large variation in bridging anticoagulation use for AF exists between specialists. A history of stroke has more influence on bridging anticoagulation use than an absolute stroke risk. How standardized protocols may promote more evidence-based practice remains to be determined.