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Can We Measure the Individual Prothrombotic or Prohemorrhagic Tendency by Global Coagulation Tests?

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Abstract

Hemostasis is a complex process in which abnormalities can cause shifts toward prothrombotic or prohemorrhagic states resulting in thrombosis or bleeding, respectively. Several coagulation tests may be required to characterize these defects but may yet not always reflect a patient's true hemostatic capacity. Thus, global coagulation tests aiming to simulate the coagulation process in vitro instead of measuring single components thereof are certainly of interest to assess prothrombotic or prohemorrhagic tendencies. This review describes the development and application of global coagulation tests, concentrating on the more widely used methods of viscoelastometry and thrombin generation. A focus is placed on conditions characterized by simultaneous changes of various components of hemostasis, such as anticoagulant therapy or hormone-induced coagulopathy, in which global coagulation tests are especially promising. If the key challenges of standardization and automation of these tests are solved, as is the case with automated thrombogram or clot waveform analysis, global coagulation assays will play an important role in the future of laboratory diagnostics of hemostasis and thrombosis.

Keywords
- global coagulation tests
- hypercoagulability
- bleeding
- anticoagulant drugs
- hormone-induced coagulopathy

Zusammenfassung

Die Hämostase ist ein komplexer Prozess, bei dem Anomalien prothrombotische oder prohämorrhagische Zustände auslösen können, die zu einer Thrombose oder Blutung führen. Die Diagnose solcher Störungen kann mehrere Labortests erforderlich machen, die dennoch nicht immer die tatsächliche Hämostasekapazität eines Patienten widerspiegeln. Daher zielen Globalteste der Gerinnung darauf ab, den Gerinnungsprozess in vitro zu simulieren, anstatt einzelne daran beteiligte Komponenten zu messen. Diese Übersichtsarbeit beschreibt Entwicklung und Einsatz dieser Globalteste, wobei sie sich auf die verbreiteten Methoden der Viskoelastometrie und Thrombingenerierung konzentriert. Im Fokus stehen Zustände mit gleichzeitigen Veränderungen verschiedener Hämostasekomponenten, etwa Antikoagulanzientherapie oder Hormon-induzierte

Schlüsselwörter
- Globalteste der Gerinnung
- Hyperkoagulabilität
- Blutung
- Antikoagulanzien
- Hormon-induzierte Koagulopathie

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Koagulopathie, bei denen der Einsatz von Globaltesten besonders vielversprechend ist. Falls es, wie beim *Automated Thrombogram* oder der *Clot Waveform Analyse*, gelingt, die zentralen Herausforderungen der Standardisierung und Automatisierung zu lösen, werden Globalteste der Gerinnung eine wichtige Rolle in der Zukunft der Labordiagnostik von Hämostase und Thrombose spielen.

Introduction

Global coagulation tests aim at measuring the clotting system in its entirety, instead of focusing on an individual protein or pathway.[1,2] The definition of "global assay" is difficult to delimit. It could be said that they are function tests of the hemostatic system, where assay conditions are chosen to reflect the interaction of all its components in the same way as they would in vivo. Current guidelines incorporate the use of global coagulation tests especially in the management of acute hemorrhage.[3,4] Various approaches for global coagulation testing are available, among which the following examples will be briefly introduced below: viscoelastometric testing, clot waveform analysis (CWA), thrombin generation assay (TGA), and sonic estimation of elasticity via resonance (SEER).

Viscoelastometric coagulation testing by forced oscillation rheometry was first presented in 1948.[5] By capturing clot formation, clot elasticity development, and fibrinolysis in real time, it mainly reflects the coagulation process in terms of maximal fibrin clot formation.[6] It has become a method with a broader range of applications since the 1970s as it is convenient as a point-of-care test.[7,8] In the 1980s the method was used for monitoring hemostasis during liver and cardiac surgery.[9,10] The currently most widely applied test systems are thromboelastography (TEG) and rotational thromboelastometry (TEM). A recent variant of TEG, TEG-6s, applies resonance-frequency viscoelasticity measurements and premixed disposable multichannel microfluidic cartridges to bypass the limitations of prior models. This point-of-care device can provide the measurements on whole blood and eliminates the need for centrifugation, which is a gain of time and also reflects the interaction between coagulation pathways and cellular blood components.[11] The Sonoclot is also considered a viscoelastrometric coagulation test and consists of a device which measures the changing impedance to movement imposed by the developing clot on a small probe vibrating at an ultrasonic frequency in a clotting blood sample.[12] Not so far away from the viscoelastometric methods, sonorheometry is a novel method, commercialized under the brand name Hemosonics Quantra (Diagnostica Stago), that has recently been authorized on the European and U.S. markets. The technology is based on the utilization of high-frequency ultrasound pulses to quantify the shear modulus (i.e., stiffness) of a blood sample during the process of coagulation. The shear modulus is a parameter that describes the elastic properties of a solid material.[13,14]

CWA differs from other global coagulation assays as it is an enhanced version of a global clotting time. First described in 1997, it makes use of photo-optical measurement of the clotting-induced change in transmittance/absorbance over time.[15] Initially it was developed to detect and to monitor disseminated intravascular coagulopathy (DIC). It has been proven to be a highly specific and sensitive assessment tool and is therefore recommended by the guidelines for diagnosis and treatment of DIC.[16,17] Some authors made use of the CWA to assess coagulation abnormalities in septic patients and suggested that CWA even outperforms standard inflammation parameters in the determination of the severity and prognosis of sepsis.[18] However, CWA is not very sensitive toward slight thrombogenic states, like hormone-induced coagulopathy. An evolution of this test led to the FibWave, a new method based on the same principle as CWA, which seems to be more sensitive toward these slight changes in coagulation factor levels.[19]

The measurement of in vitro thrombin generation in whole blood and plasma was first described in 1953.[20,21] The initially time-consuming method was then modified and refined over the following decades leading to several thrombin-generation platforms.[22,23] In general, they evaluate in vitro thrombin generation in a sample of platelet-poor plasma after coagulation activation by tissue factor (TF) and phospholipids, continuously monitoring the reaction of thrombin generation by means of a thrombin-specific fluorogenic substrate (or eventually a chromogenic one). Some TGA variants may also be performed in platelet-rich plasma and even in whole blood and may therefore reflect the interplay of these cellular components and the coagulation proteins.[24] At this time, TGA is recommended for the assessment of activated protein C (APC) resistance.[25,26] Indeed, by adding exogenous APC, this assay is capable of detecting changes in hemostasis induced by the hormonal status of women (i.e., during pregnancy, on hormonal contraceptive or hormonal replacement therapy [HRT] during menopause). Moreover, it is also sensitive to thrombophilia such as factor V Leiden (FVL) mutation, prothrombin 20210G > A mutation, or protein S (PS) deficiency.[27] Therefore, this TGA variant, termed endogenous thrombin potential (ETP)-based APC resistance assay, may provide sufficient information to screen several losses or gains of function which increase the risk of thrombosis. (The ETP-parameter, which represents the amount of thrombin generated after in vitro activation of coagulation, is one of the five TGA-parameters.) Recently, a validated ETP-based APC resistance assay and a harmonized scale have been proposed to consider the use of this test in clinical routine in view of its screening potential.[27] The most widely used TGA method, the calibrated automated thrombogram (CT), is performed in a 96-well

plate, and it requires specialized technologists. This has resulted in a low implementation of this technique in routine laboratories but recent evolutions of TGA platforms have led to the advent of an automated system, the ST-Genesia, which should resolve this issue.[28]

Assessment of Prothrombotic Abnormalities and Complex Coagulation Disorders

Global coagulation times, while being sensitive in the screening for deficiencies of coagulation factors, utilize high amounts of activators to initiate clotting, which substantially reduces their sensitivity to detect small quantities of coagulation-activating factors in the circulation. Nevertheless, several studies have shown an association between a shortened activated partial thromboplastin time (aPTT) and the risk of recurrent venous thromboembolism (VTE).[29–31] However, activity levels of the determinants of the aPTT, coagulation factors VIII, IX, and XI, have been shown to be better predictors of recurrent VTE,[31] and no predictive value of the aPTT has been found regarding the thrombotic risk associated with trauma, surgery, or cancer.[32–34] CWA takes into account not only the clotting time but also curve changes of the optical density. This further developed variant of the aPTT has been predominantly used to investigate abnormalities in complex coagulation disorders, and certain characteristics of CWA parameters have been shown to be predictors of hypercoagulability in sepsis or of VTE in patients with liver cirrhosis.[35–37]

Viscoelastometric methods, including TEG, TEM, and Sonoclot, have found widespread use to guide the therapy with blood products in patients with active bleeding. Changes of parameters of both tests, especially an increase of the maximum amplitude (TEG) or maximum clot firmness (TEM), have been suggested to be indicative of a hypercoagulable state.[38,39] The ability of viscoelastometric testing to predict clinical thromboembolic events was recently analyzed in a large meta-analysis that included 41 studies with more than 10,000 patients, including predominantly trauma patients, patients undergoing elective surgery, patients with malignancies, and patients with a history of arterial or venous thrombosis. This meta-analysis reported a moderate ability of TEG and TEM to discriminate between patients who developed thromboembolism and those who did not, with a pooled sensitivity of 56%, a specificity of 76%, and a diagnostic odds ratio of 3.6.[40] Further studies reported an association between changes of TEG or TEM parameters and situations of increased thrombotic risk, such as cancer[41,42] or pregnancy,[43–45] without investigating potential clinical manifestations of a hypercoagulable state. Initial research found the Sonoclot of some interest in cardiac and liver surgeries as well as in the assessment of hypercoagulable states.[46–48] However, the Sonoclot has not been widely adopted and there is a paucity of data regarding the reference ranges that are needed to guide clinical decisions.[49] There is also a paucity of studies that directly compare parameters of viscoelastometric testing with established molecular biomarkers of coagulation activation to predict thromboembolic events. However, in complex coagulation disorders viscoelastometric tests have the advantage of considering changes of cellular and fibrinolytic components, which are not captured by conventional plasmatic coagulation tests. While prolonged clotting times and reduced plasma levels of procoagulant factors suggest a hypocoagulable state in sepsis or liver disease, viscoelastometric parameters can be normal or even indicative for a hypercoagulable state, and might thereby better reflect a shift of the hemostatic balance in these coagulation disorders.[50–52] It has been shown that in septic patients with prolonged prothrombin time but normal or hypercoagulable parameters of viscoelastometric testing, invasive procedures are not associated with an increased bleeding risk.[53,54] This is also reflected by the discordance that has been observed between international normalized ratio and TEG R times in previous studies.[55] Also, both hyper- or hypofibrinolysis can be detected by viscoelastometric testing in trauma patients and patients with sepsis, DIC, or liver disease.[56–59] However, appropriate methodologies and reagents are required to assess these hypofibrinolytic states.[60]

The relationship between in vitro thrombin generation and thrombotic risk was investigated in several studies, in which an association between the ETP and other thrombin generation parameters and the risk of recurrent VTE was observed.[61–63] An increased ETP in platelet-rich plasma was reported in young stroke patients.[64] In another study, an increase of ETP and thrombin peak height was associated with the risk of acute ischemic stroke but not with coronary heart disease in elderly patients.[65] Among the classical thrombophilia risk factors, deficiencies of antithrombin (AT), PS (in a modified version of the assay in which thrombomodulin is added), and the FVL and prothrombin 20210G > A mutations are associated with an increased ETP.[66–69] An increased ETP has also been observed in the presence of acquired risk factors of thrombosis, including cancer, the use of combined hormonal contraceptives (CHCs), and pregnancy.[70,71] However, a correlation between the increase of in vitro thrombin generation and indirect markers of in vivo thrombin formation was not observed in these studies. The TGA has been found to be sensitive to various direct-acting agents of coagulation in the analyzed plasma including microparticles, TF, and lipopolysaccharides.[72–74] By adding an amount of exogenous APC, the TGA can be used to assess the functionality of the anticoagulant protein C (PC) pathway. Thrombin generation tests used in this variant are capable of detecting hereditary APC resistance (e.g., FVL mutation) as well as acquired forms of impaired APC sensitivity (e.g., the one caused by ligands of estrogen receptors such as the estrogen components of CHCs and other drugs).[75–77]

Assessment of Hormone-Induced Coagulopathy

CHCs and postmenopausal HRTs are widely used around the world. More than 200 million women aged between 14 and 60 years are undergoing one of these treatments, which are associated with a risk of thrombosis that affects nearly 100,000 women each year.[78–80] CHCs were first introduced on the market in the early 1960s and have been extensively

Table 1 Risk of developing venous thromboembolism (VTE) in women using combined hormonal contraceptives (CHCs), adapted from European Medicines Agency[83]

Generation of used CHC (progestin and derivative)	VTE risk/year
No CHC use and no pregnancy	2 out of 10,000 women
Second (levonorgestrel, norethisterone, or norgestimate)	5–7 out of 10,000 women
Third (desogestrel or gestodene)	9–12 out of 10,000 women
Fourth (drospirenone)	9–12 out of 10,000 women

studied since then.[81,82] Overall, it has been revealed that the effect of CHCs on hemostasis depends on the type and dose of estrogen, and the type and dose of the associated progestogen, which is clinically reflected by the estimated risk of VTE depending on the different generations of CHCs (►**Table 1**).[83]

Although the thrombogenicity of CHCs is well known, current practice does not regularly include a laboratory screening to assess a woman's individual risk of VTE before initiation of CHC use or HRT. While the prescribing physician's decision considers the patient's wish and her family history of thrombosis, thrombophilia risk factors (e.g., the FVL and prothrombin 20210G > A mutations, and deficiencies of AT, PC, and PS) are generally not taken into account, although they lead to a higher baseline risk of VTE.[84,85] Among these genetic risk factors, FVL and prothrombin 20210G > A are the most frequent and are present in 3 to 15% of the Caucasian population.[86] The risk of a first VTE event is four- to eightfold higher in heterozygous carriers while it may reach a relative risk of 30 to 80 in homozygous carriers.[87–90] When combined with the use of CHCs or HRT, it affects the coagulation cascade synergistically leading to a major risk of thrombosis during the first year of use.[85,91–96] For example, if it is reported that the relative risk of thrombosis in heterozygous FVL carriers is approximately 4, in women on third-generation CHCs, it will be approximately 3.5 and the combination of these two risk factors leads to a relative risk of 45, revealing a synergistic index of more than 3.[97]

Overall, CHCs and HRTs induce changes in numerous hemostasis variables, depending on their estrogenic and progestin compounds. On one hand, they impact positively the procoagulant pathways (i.e., increased levels of fibrinogen, prothrombin, factors VII, VIII, and X) and, on the other hand, they impact negatively the anticoagulant pathways (i.e., decreased levels of AT, PS, and TF pathway inhibitors). These changes also lead to an acquired APC resistance, which is an independent risk factor of VTE.[78,98,99] Today, a complete thrombophilia screening requires several coagulation tests which can make the interpretation of the results difficult and expensive. Even if the changes of coagulation factor levels induced by CHCs do not exceed their respective normal ranges, an increased thrombogenicity is the result of a synergistic effect of these changes and global tests are able to reveal these synergistic effects. It should be noted that changes are more pronounced with third- and fourth-generation CHCs in comparison to second-generation CHC, which corresponds to the clinical risk of VTE observed in epidemiological studies.[92,100,101] Moreover, the endpoint of clotting-time-based assays corresponds to the beginning of thrombin generation, which means that the conventional tests inform only on the initiation phase of coagulation but not on the hemostatic capacity in terms of clot formation and maximum thrombin generation.[102] Therefore, a global coagulation assay would seem more appropriate to assess the overall thrombotic risk in women on CHC or HRT treatment. Such assessment could be informative before the initiation of any hormonal therapy or to ensure a longitudinal monitoring since interindividual variability in response to the treatment has been reported, corresponding to the interindividual variability in the metabolism of ethinylestradiol.[103]

TEG has been assessed in women on CHCs. In the study of Sucker et al the influence of oral contraception on TEM was investigated in a small group of women and significant changes of several parameters including shorter clot formation time (upon extrinsic activation), broader maximum clot firmness (upon intrinsic activation), and broader *a*-angle (upon extrinsic activation) were observed.[104] However, the study size was limited and therefore, further investigation is required before confirming that the TEM may be appropriate to assess hemostasis changes induced by CHCs. Thrombin-generation testing has been found to be very sensitive toward hemostasis changes induced by CHCs. A correlation between the increase of the normalized APC sensitivity ratio (nAPCsr, the measure of the ETP-based APC resistance) and the risk of VTE has been shown in different studies demonstrating the high potential of ETP-based APC resistance testing for the prediction of VTE risk both in the presence and absence of the FVL mutation.[27,105,106] In addition, the extent of the increase of the nAPCsr has been found to be associated with the VTE risk observed in epidemiological studies (►**Fig. 1**).[107] Interestingly, the nAPCsr and the relative risk of VTE in patients with heterozygous FVL and in women on third-generation CHCs are similar suggesting a close association between this test and the relative risk of VTE. Also, the combination of estradiol valerate with dienogest, which demonstrated the lowest VTE risk, even when compared with levonorgestrel-containing products, demonstrated a lower nAPCsr, as depicted in ►**Fig. 1**.[107] Thus, identification of women with a higher thrombotic risk before the initiation of hormonal therapy would allow two opportunities: first, to suspect an underlying genetic disorder and thus to guide toward a more specific diagnostic test and life-long prevention strategy, and second, to prescribe the safest hormonal therapy according to the patient's clinical status. In addition, it would

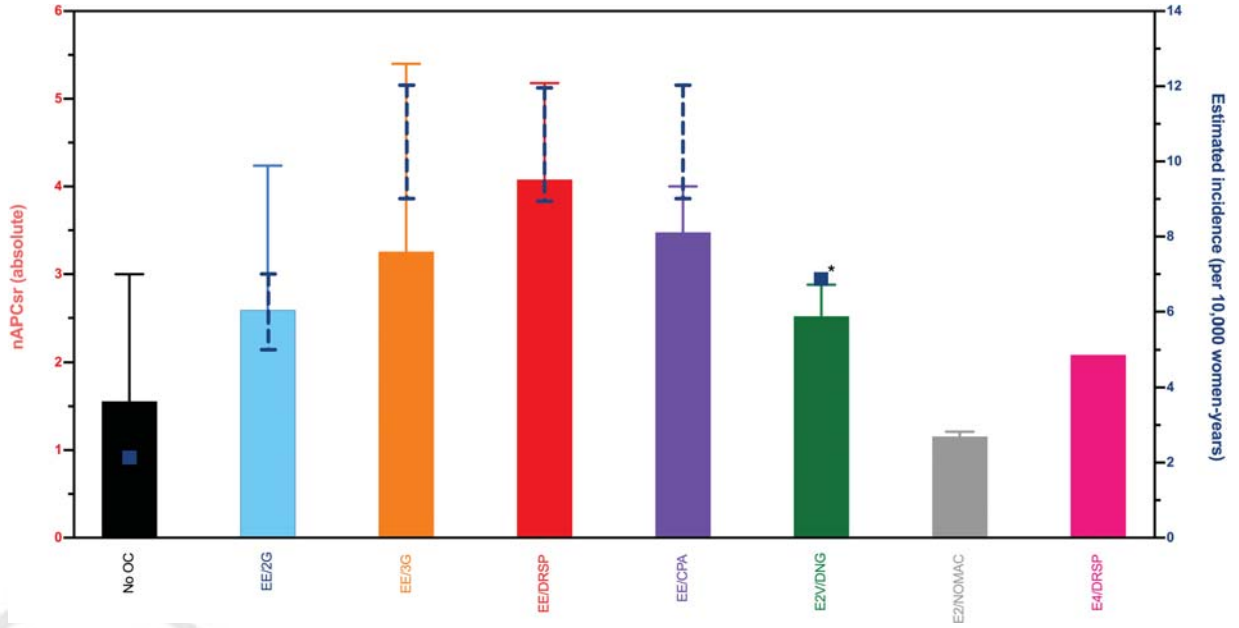

Fig. 1 Synthesis of studies from 1997 to 2019 investigating the impact of oral contraceptives on the APC resistance, when expressed as nAPCsr (absolute). Left *Y*-axis represents nAPCsr in absolute values. Right *Y*-axis represents the estimated incidence of venous thromboembolism issued from the EMA assessment report.[83] The estimated rates of VTE is 2 per 10,000 women-years in non-OC users; 5 to 7 in second-generation CHC users; 9 to 12 in third-generation, drospirenone and cyproterone acetate CHC users; and around 7 in dienogest CHC users (data from the INAS-SCORE study; direct comparison should not be made since in this study the combination of ethinylestradiol with levonorgestrel was 8.8/10,000 women-years). The lower risk of estradiol valerate plus dienogest compared with other CHCs including ethinylestradiol plus levonorgestrel observed in the INAS-SCORE study is associated with a lower impact of this combination on the nAPCsr. Estetrol (E4) plus drospirenone appears to have a low impact on nAPCsr suggesting that the risk is linked to the estrogenic component rather than to the progestin.[107] 2G, second-generation combined oral contraceptive; 3G, third-generation combined oral contraceptive; CHC, combined hormonal contraceptive; CPA, cyproterone acetate; DNG, dienogest; DRSP, drospirenone; EE, ethinylestradiol; E2, 17β-estradiol; E2V, estradiol valerate; EMA, European Medicines Agency; ETP, endogenous thrombin potential; nAPCsr, normalized activated protein C sensitivity ratio; NOMAC, nomegestrol acetate; OC, oral contraceptive; VTE, venous thromboembolism.

support the implementation of risk minimization strategies to reduce the life-long risk of VTE.

Assessment of Prohemorrhagic Tendencies

Both commonly applied viscoelastometric tests (TEG and TEM) have been shown to be eligible for the detection of coagulopathy and hemorrhage in trauma, surgery, and beyond that, guiding hemostatic therapy in adult and pediatric patients.[108–110] TEG has been demonstrated to improve the treatment of acute hemorrhage in terms of a decreased amount of transfusions and lowered costs.[111] In vitro thrombin generation measurement is indicative for hypercoagulability but might also become an important tool in managing hemorrhage.[112] It has been proven useful in the treatment of hemophilia patients, especially when bypassing agents are applied.[113,114] In vitro thrombin generation has been shown to be reduced in hemophilia A and B as well as in rare congenital coagulation factor deficiencies including deficiencies of factor II, V, VII, X, XI, and XII.[115] An ETP below 20% of its normal value was associated with an increased risk of bleeding in patients with hemophilia A or B.[116] While factor XIII (FXIII) deficiency did not affect thrombin generation, some data may reveal that this may reduce the maximum amplitude of the TEG.[117] However, these data were obtained using commercially available plasma from patients with severe FXIII deficiency. Patients with mild to moderate FXIII deficiency may not present with the same extent of changes in TEG. Further data are needed to confirm the usefulness of TEG in this context.

Factor replacement therapy in hemophilia patients with and without inhibitors can also be monitored by thrombin generation tests.[118–120] Treatment monitoring and estimation of bleeding tendency in hemophilia patients is also another possible application of CWA.[121,122] TGA might give more information about the hemostatic capacity in total than viscoelastometric testing as it looks beyond fibrin clot formation. TEG and TEM have also been shown to be useful in the management of patients with acquired forms of coagulopathy in major surgery.[123]

Assessment of Anticoagulant Therapies

A monitoring of the anticoagulant activity of direct oral anticoagulants (DOACs) is generally not necessary but a point estimation could be useful in vulnerable patients.[124] Specific laboratory tests have been pointed out as the more appropriate assays since they provide results expressed in ng/mL, which corresponds to the unit used for the definition of the tentative thresholds associated with bleeding risks or particular interventions (i.e., administration of antidote, eligibility for thrombolysis, etc.).[125–130] However, these thresholds are, for some of them, arbitrary, based on expert's opinions and may not reflect the intrinsic anticoagulant activity of DOACs.

For example, the threshold proposed for the administration of reversal agents does not consider the different pharmacodynamic profiles of the drugs.[126] Namely, it has already been demonstrated that 30 or 50 ng/mL of rivaroxaban does not have the same anticoagulant activity as the same amount of apixaban, betrixaban, or edoxaban (►Fig. 2).[131–135] This is also reflected by the necessity of adapting the methodology of specific chromogenic anti-Xa assays depending on the drug used.[135] Consequently, these tests do not reflect the in vivo intensity of anticoagulant activity. TGA, viscoelastometric assays (TEG, TEM, ClotPro) and more recently SEER sonorheometry are considered as global assays of hemostatic function.[121,124] They are able to measure the kinetics of thrombin or fibrin formation over time in clotting plasma.[102] These assays provide more information than simple clotting time tests and are of interest for the detection of coagulation abnormalities.[136] Nevertheless, in the setting of anticoagulant therapy, most of these global assays often lack sensitivity or if they are modified to become specific, i.e., by the addition of particular triggering agents, they no longer provide a global assessment as this is the case with the ecarin TEG for specific dabigatran assessment. Indeed, they only focus on a particular pathway or factor. Activation of coagulation factors of the common pathway by snake venom or addition of direct catalytic enzymes like activated factor X or thrombin is frequent in this specific testing, and therefore retroactivation pathways or contribution of upstream coagulation factors is lacking. However, this may help to discriminate an underlying coagulopathy not secondary to the effect of the anticoagulant therapy (i.e., acquired hemophilia) that could also result in bleeding.

Several in vitro and ex vivo studies have already demonstrated the potential of TGA for the assessment of the effect of DOACs and the monitoring of reversal therapies without modifying the inducers or the reagents, meaning that the test keeps its capacity to assess the coagulation in its globality.[130–133,137–141] Preliminary observations showed that thrombin generation testing is affected by all anticoagulant drugs and therefore it could be the candidate assay.[140,142–146] The test has been found to be very sensitive to all kinds of anticoagulants[130–133,138,143,147] and may better represent the interindividual response than just exploring plasma concentrations.[140] In addition to considering the interindividual response to an antithrombotic drug, thrombin generation testing is also able to explore in more detail the impact of anticoagulants on the coagulation process. Namely, depending on the type of drug, different studies confirmed that the fingerprint of in vivo thrombin generation differs, revealing the different pharmacodynamics of the drugs (►Fig. 2).[141,143,147,148] This is of particular importance since bleeding or thrombosis has been reported within the "on-therapy" range, demonstrating that the drug level alone may not be sufficient to identify those who are more at risk.[149] However, further investigation on patients who bleed or who have recurrent thrombosis while on a fixed dose of anticoagulants is needed to show the benefit of in vitro thrombin generation testing and provide cut-offs for bleeding and thrombotic complications. The TGA has also been reported to be an informative tool to document on antidote administration in polytrauma models with direct implication for patient care.[150] This is particularly important as it may help in adjusting the dose of prothrombin complex concentrate to administer.[151]

Limitations and Open Questions

Despite their advantages, global coagulation assays still remain experimental in vitro models of hemostasis that only aim to mimic all important aspects of the physiological (and pathological) clotting process. To date, none of the existing global assays has been shown to actually involve all components of hemostasis. For example, the major shortcoming of the viscoelastometric methods TEG and TEM are their insensitivity to platelet dysfunction and von Willebrand factor deficiency. FXIII, which is also not assessed in the global clotting time assays, is not adequately reflected, too.[123] TGAs can be modified to diagnose defects in fibrinogen, fibrinolysis, and the PC system.[75] However, these variations detract from the paradigm of a global coagulation assay and may dilute their advantages. No coagulation test will be able to predict an increased risk of bleeding or

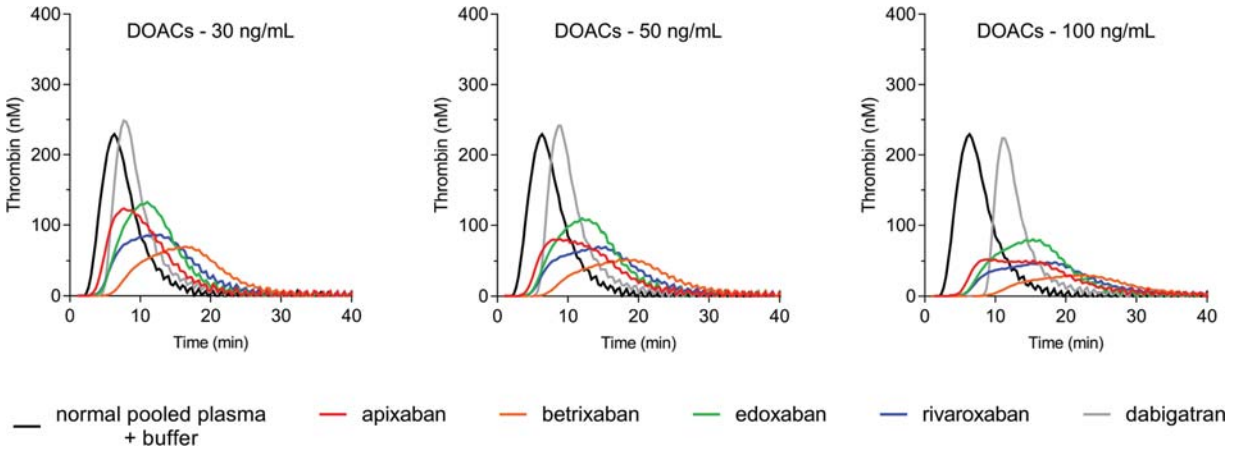

Fig. 2 Thrombin-generation profiles of different direct oral anticoagulants (DOACs) at thresholds used for antidote administration in case of bleeding or urgent surgery and to allow thrombolysis (i.e., 30, 50, or 100 ng/mL).

thrombosis due to factors affecting the vasculature or other tissues as well as compromised cellular or plasmatic coagulation. If the origin of a bleeding is not initially caused by compromised hemostasis, all global coagulation tests can be perfectly normal.

One particular problem of global coagulation assays is preanalytics as their high sensitivity makes them vulnerable to inaccuracies and variables of sample collection and preparation.[152,153] On the other hand, viscoelastometric testing lacks the sensitivity to detect patients with thrombophilic defects, which is essential for its use, e.g., in the screening of women before the initiation of a hormonal therapy.[154,155] Viscoelastometric assays also have a poor to zero correlation with platelet related/associated defects like Glanzmann's thrombasthenia or von Willebrand disease. On a more technical side, especially with point-of-care tests, in which whole blood is investigated, standardization is often lacking. There are also difficulties for laboratories to verify the performance of these methods. Another significant limitation of the viscoelastometric assays is the requirement for stabilized surfaces to avoid false pin/cup movements. Current research is ongoing to develop mobile viscoelastometric measuring devices. The lack of standardization between the different technologies on the market[23] also limits the routine clinical use of TGA, which has been addressed in several studies.[152,156,157] Recently, the ST-Genesia, a fully automated thrombin generation analyzer, was reported to provide enhanced reproducibility compared with the CT. This new analyzer also offers a normalization of TGA parameters with the use of CE-marked reference plasma, calibrators, controls, and reagents that minimize the interlaboratory variability.[28,158,159] While TEG and TEM have the major advantage of being suitable to be used point-of-care, the main disadvantage of thrombin generation tests is their comparably long turnaround time, which may be reduced by testing whole blood.[160]

In the assessment of the individual prothrombotic or prohemorrhagic tendency, global coagulation tests have to compete with tests that detect specific defects, such as deficiencies of coagulation factors or inhibitors, and in the assessment of hypercoagulability also with tests that measure specific markers of coagulation activation. In the field of complex coagulation disorders these alternative approaches have the disadvantages that they cannot be performed point-of-care, and that possibly a lot of different specific tests need to be performed to get the same information as with a global point-of-care assay, tests that require more blood sampling and a more specialized laboratory with a broad range of methods. Furthermore, a complex coagulation disorder will be associated with a multitude of changes of specific coagulation tests complicating their interpretation. In addition, indirect markers of thrombin formation are indicators of events that have taken place in the past, as active thrombin itself is cleared from the circulation within minutes.[161] The interpretation of activation markers of coagulation and fibrinolysis is further impeded by a high variation of their residence in circulation.[162] However, in other very common indications of coagulation testing, such as the assessment of the bleeding risk before elective surgeries or the risk of first or recurrent thrombosis, the aforementioned advantages of global coagulation tests do not apply. Although many studies cited in this review measured specific coagulation parameters or activation biomarkers in addition to global coagulation testing, there is a paucity of studies directly comparing global coagulation assays with the standard of care in terms of clinical outcomes. A summary of specific advantages and limitations of TEG/TEM, CWA, and TGA is provided in ►Table 2.

Ongoing Research

Beyond the assay techniques presented so far, several other global coagulation assays have been developed, either variations of existing tests such as rheometric assays other than TEG or TEM,[163] or assays based on novel principles, some of which might find a role in clinical routine in the future but at the current stage of their development further research is required. Among these methods are the thrombodynamics assay, simultaneous measurement of thrombin and plasmin generation, the observation of clot formation in flow perfusion chambers, and artificial endothelium testing platforms. The concept of global testing is here taking a bit forward the process to evaluate the interaction between cellular and plasmatic components with surfaces. In the thrombodynamics assay spatial fibrin formation in plasma is monitored by videomicroscopy after being triggered by immobilized TF, with a clot initially forming on the activator and then propagating into plasma (similar to the in vivo process).[164] The temporospatial formation of thrombin can be monitored parallel to that of fibrin.[165] Separation of the phases of

Table 2 Summary of relative advantages and limitations of thromboelastogram (TEG), thromboelastometry (TEM), clot waveform analysis (CWA), and thrombin generation assay (TGA)

Assay	Advantages	Limitations
TEG/TEM	• Point-of-care analysis • Sensitive to abnormalities of fibrinolysis	• No detection of platelet-related disorders • Poor standardization
CWA	• Could be performed on routine coagulation analyzers	• Low sensitivity toward mild thrombogenic states
TGA	• Highly adaptable to assess different thrombophilic states • Comparably high degree of standardization and automation	• (Personnel-) and time-intensive

activation and propagation is associated with a high sensitivity of the assay to the presence of direct activators of coagulation in plasma, such as circulating TF or activated factor XI.[166,167] Hypercoagulability measured using the thrombodynamics assay has shown an association with elevated D-dimer levels in patients with sepsis.[168] Several methods of simultaneous measurement of thrombin and plasmin generation have been developed, in which coagulation activation is triggered by TF, calcium, and phospholipids or small amounts of exogenously added thrombin while fibrinolysis activation is initiated by the addition of tissue-type plasminogen activator. These methods have been evaluated in various patient populations with known hyper- and hypocoagulable states and allow for the assessment of the fibrinolytic system which is not captured by conventional TGA.[169–171] Flow chambers, in which the formation of platelet and fibrin clots can be observed by microscopy, are increasingly used to monitor the combined processes of platelet aggregation, thrombus formation, and coagulation in human blood, allowing high-throughput measurement of platelet activation processes, even in small blood samples.[172] Several studies have demonstrated the potential of flow perfusion chambers to detect prothrombotic changes in blood.[173–175]

As mentioned earlier, TGA is designed to estimate the thrombin concentration over time which is, however, not the final endpoint of the coagulation process. The assessment of fibrin formation by assays and analyzers able to visualize the kinetic formation of fibrin clots is interesting. Usually, clotting assays only report clotting time but many other kinetic parameters may be relevant and have already demonstrated their usefulness in the diagnosis and the prognosis of different coagulation abnormalities.[120] Recently, the FibWave, a newly designed coagulation assay based on the analysis of the kinetics of fibrin clot formation, assessed the overall coagulation process by measuring changes in light absorbance that occur during clot formation.[176] This test appears to be sensitive, faster, and less expensive than TGA in the assessment of anticoagulant properties. Thanks to its ease of use, its possibility to be implemented on routine coagulometers, and its capacity to assess the whole coagulation process, the FibWave could provide the clinicians with a global coagulation test, sensitive at relevant threshold concentrations with a reproducibility similar to the one observed on the CT system.[177]

Viscoelastometric testing has been investigated for monitoring as well as differentiating between classes of DOACs. Dias et al found that in the TEG 5000 dabigatran increased the R parameter of the citrated kaolin assay, and that apixaban, rivaroxaban, and dabigatran increased the activated clotting time parameter of the citrated RapidTEG.[178] Other groups found that the EXTEM clotting time could be used to detect each of the four DOACs tested; however, sensitivity was poor at drug concentrations within the therapeutic ranges.[179] Recently, the TEG 6s has been assessed for the monitoring of DOACs.[180] It was demonstrated that the R parameter was the most sensitive and correlated with the DOAC concentration when assessed via the specific cartridge for factor Xa or thrombin inhibitors.[180] The predictive value of this assay was reported to be very high (>98%), which is particularly important in emergency situations.

A novel approach to overcome the limitations of conventional coagulation activation marker measurement and, in some sense, an in vivo counterpart of in vitro thrombin generation by TGA is the measurement of active key enzymes of hemostasis. Using highly specific aptamers that do not cross-react with the inactive proenzymes, as capture ligands, enzyme capture assays have been developed that allow direct quantification of free thrombin and APC in human plasma in the picomolar range.[181,182] These oligonucleotide-based enzyme capture assays have been shown to be able to measure in vivo thrombin and subsequent APC generation in real-world conditions of coagulation activation such as surgical trauma or septic shock.[183,184] In addition, these assays have been applied in human models of venous stasis or coagulation activation induced by activated factor VII.[185,186] The latter approach, termed stimulated hemostasis activity pattern analysis (SHAPE), revealed distinctive reaction patterns of pro- and anticoagulant responses in carriers and noncarriers of the thrombophilia FVL and prothrombin 20210G > A mutations and in FVL mutation carriers with and without a history VTE.[187] While these data have shown the ability of the SHAPE approach to assess the functionality of the thrombin–PC pathway, its ability to predict first or recurrent VTE in the future is still under investigation.

As a final remark, in the current digital era, real-time remote viewing of any of the here presented global methods would be of interest for clinicians, as it will permit to directly monitor the results in real-time while facing the patients.

Time Capsule

- Since the first introduction of global coagulation assays in the 1950s several commercially available assays have found broad use, including thromboelastography and the thrombin generation assay.
- The advantages of these assays lie in the assessment of simultaneous pro- and anticoagulant changes occurring, e.g., in complex coagulation disorders or under anticoagulant treatment.
- Global thrombophilia screening is needed to assess the thrombogenicity before the induction of some thrombogenic therapies. Global coagulations tests are candidate assays for this screening.
- Standardization and automation are key factors to improve global coagulation assays. Automated thrombin generation is promising regarding screening of both hemorrhagic and thrombotic tendencies.
- A perfect in vitro simulation of hemostasis might remain an impossible task as factors outside of the coagulation and fibrinolysis system contribute to an increased risk of bleeding or thrombosis.

Authors

Jonathan Douxfils

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After being graduated in pharmaceutical sciences in 2011, Prof. Jonathan Douxfils obtained his PhD in biomedical and pharmaceutical sciences in 2015. In 2018, he gained an academic position at the University. The research studies directed by Prof. Douxfils played a leading role in the establishment of guidelines for the laboratory measurement of DOACs in the routine setting. These recommendations have been used so far by several expert societies involved in the field of thrombosis and hemostasis. He also exercises his expertise as a pharmacovigilance expert at the European Medicines Agency, is co-chairmen of the SSC Control of Anticoagulation at the International Society of Thrombosis and Haemostasis (ISTH), and is also the co-founder and the CEO of QUALIblood, a contract research organization (CRO) aiming to provide the industries, hospitals, and universities with all the analytical services for blood investigations and hemocompatibility testing. In 2019, he received the Eberhard F. Mammen Young Investigator Award for his research on the development of a new algorithm based on thrombin generation to assess hormone-related prothrombotic changes.

Passionate about clinical and laboratory research, he is involved in several projects aiming to solve pharmacological and/or epidemiological problems especially in the field of thrombosis and hemostasis. Thanks to his translational view of the pharmaceutical and medical-device market from basic research to postmarketing pharmacovigilance, his collaborations with key opinion leaders and also with field practitioners, Prof. Jonathan Douxfils puts its expertise and know-how at the services of projects aiming to improve the safety and effectiveness of therapeutic agents to promote public health.

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After studies in medicine, economics, and management at the University of Marburg, Heiko Rühl obtained his license to practice medicine and his MD in 2005. He started his medical specialist training at the University Hospital Gießen and completed it at the University Hospital Bonn in 2012, where he gained a position as a senior physician at the Institute of Experimental Hematology and Transfusion Medicine in 2012. In 2015 he became Medical director of the MVZ Venusberg GmbH, the medical care center of the University Hospital Bonn. Since 2016 Dr. Rühl has been a holder of a scholarship of the Stiftung Hämotherapie-Forschung (Hemotherapy Research Foundation). In 2017 he completed his Habilitation on the characterization of hypercoagulable phenotypes at the University of Bonn.

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Sara Reda obtained her license to practice medicine in 2009 and started her medical specialist training in Internal Medicine/Cardiology at the University Hospital of Cologne, Germany. She completed her training in 2015 at the University Hospital of Salzburg, Austria. She then joined the Institute of Experimental Hematology transfusion medicine at the University Hospital Bonn in 2017 and will complete her specialist training in transfusion medicine as well as hemostaseology in 2020. Her research interests include the genotype–phenotype correlation of hereditary

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Conflict of Interest

Among the authors, J.D. is chief executive officer and founder of QUALIblood and reports personal fees from Diagnostica Stago, Roche, Roche Diagnostics, Daiichi-Sankyo, and Portola, outside the submitted work. The other authors have no conflicts of interest to disclose.

References

1 Brummel-Ziedins KE, Wolberg AS. Global assays of hemostasis. Curr Opin Hematol 2014;21(05):395–403
2 van Geffen M, van Heerde WL. Global haemostasis assays, from bench to bedside. Thromb Res 2012;129(06):681–687
3 Kozek-Langenecker SA, Afshari A, Albaladejo P, et al. Management of severe perioperative bleeding: guidelines from the European Society of Anaesthesiology. Eur J Anaesthesiol 2013; 30(06):270–382
4 Spahn DR, Bouillon B, Cerny V, et al. Management of bleeding and coagulopathy following major trauma: an updated European guideline. Crit Care 2013;17(02):R76
5 Hartert H. Blutgerinnungsstudien mit der Thrombelastographie; einem neuen Untersuchungsverfahren. Klin Wochenschr 1948; 26(37–38):577–583
6 Schols SE, Heemskerk JW, van Pampus EC. Correction of coagulation in dilutional coagulopathy: use of kinetic and capacitive coagulation assays to improve hemostasis. Transfus Med Rev 2010;24(01):44–52
7 Raviv G, Cramer DB, Epstein M, Zukerman L, Caprini JA. Computerization of three-channel thrombelastograph. J Med 1978;9 (01):33–41
8 Perry DJ, Fitzmaurice DA, Kitchen S, Mackie IJ, Mallett S. Point-of-care testing in haemostasis. Br J Haematol 2010;150(05): 501–514
9 Kang YG, Martin DJ, Marquez J, et al. Intraoperative changes in blood coagulation and thrombelastographic monitoring in liver transplantation. Anesth Analg 1985;64(09):888–896
10 Tuman KJ, Spiess BD, Schoen RE, Ivankovich AD. Use of thromboelastography in the management of von Willebrand's disease during cardiopulmonary bypass. J Cardiothorac Anesth 1987;1 (04):321–324
11 Olechowski B, Dalton RT, Khanna V, et al. Detection of individual responses to clopidogrel: validation of a novel, rapid analysis using thrombelastography 6s. Cardiovasc Ther 2018;36(04):e12433
12 Hett DA, Walker D, Pilkington SN, Smith DC. Sonoclot analysis. Br J Anaesth 1995;75(06):771–776
13 Corey FS, Walker WF. Sonic estimation of elasticity via resonance: a new method of assessing hemostasis. Ann Biomed Eng 2016;44(05):1405–1424
14 Nelb GW, Kamykowski GW, Ferry JD. Rheology of fibrin clots. V. Shear modulus, creep, and creep recovery of fine unligated clots. Biophys Chem 1981;13(01):15–23
15 Braun PJ, Givens TB, Stead AG, et al. Properties of optical data from activated partial thromboplastin time and prothrombin time assays. Thromb Haemost 1997;78(03):1079–1087
16 Bakhtiari K, Meijers JC, de Jonge E, Levi M. Prospective validation of the International Society of Thrombosis and Haemostasis scoring system for disseminated intravascular coagulation. Crit Care Med 2004;32(12):2416–2421
17 Levi M, Toh CH, Thachil J, Watson HG; British Committee for Standards in Haematology. Guidelines for the diagnosis and management of disseminated intravascular coagulation. Br J Haematol 2009;145(01):24–33
18 Dempfle CE, Lorenz S, Smolinski M, et al. Utility of activated partial thromboplastin time waveform analysis for identification of sepsis and overt disseminated intravascular coagulation in patients admitted to a surgical intensive care unit. Crit Care Med 2004;32(02):520–524
19 Evrard J, Morimont L, Siriez R, Dogne JM, Douxfils J. Assessment of the APC resistance measured by thrombin generation and clot waveform analysis: a pilot study; Special Issue: Abstracts of the XXVII Congress of the International Society on Thrombosis and Haemostasis, July 6–10, 2019. Res Pract Thromb Haemost 2019;S1:1–891
20 MacFarlane RG, Biggs R. A thrombin generation test; the application in haemophilia and thrombocytopenia. J Clin Pathol 1953; 6(01):3–8
21 Pitney WR, Dacie JV. A simple method of studying the generation of thrombin in recalcified plasma; application in the investigation of haemophilia. J Clin Pathol 1953;6(01):9–14
22 Hemker HC, Al Dieri R, De Smedt E, Béguin S. Thrombin generation, a function test of the haemostatic-thrombotic system. Thromb Haemost 2006;96(05):553–561
23 Kintigh J, Monagle P, Ignjatovic V. A review of commercially available thrombin generation assays. Res Pract Thromb Haemost 2017;2(01):42–48
24 Hemker HC, Al Dieri R, Béguin S. Thrombin generation assays: accruing clinical relevance. Curr Opin Hematol 2004;11(03): 170–175
25 Douxfils J, Morimont L, Delvigne AS, et al. Validation and standardization of the ETP-based activated protein C resistance test for the clinical investigation of steroid contraceptives in women: an unmet clinical and regulatory need. Clin Chem Lab Med 2020;58(02):294–305
26 Morimont L, Bouvy C, Delvigne AS, Dogné JM, Douxfils J. Proof of concept of a new scale for the harmonization and the standardization of the ETP-based APC resistance. J Thromb Haemost 2020; 18(04):895–904
27 Curvers J, Thomassen MC, Rimmer J, et al. Effects of hereditary and acquired risk factors of venous thrombosis on a thrombin generation-based APC resistance test. Thromb Haemost 2002;88 (01):5–11
28 Douxfils J, Morimont L, Bouvy C, et al. Assessment of the analytical performances and sample stability on ST Genesia system using the STG-DrugScreen application. J Thromb Haemost 2019;17(08): 1273–1287
29 Tripodi A, Chantarangkul V, Martinelli I, Bucciarelli P, Mannucci PM. A shortened activated partial thromboplastin time is associated with the risk of venous thromboembolism. Blood 2004; 104(12):3631–3634
30 Hron G, Eichinger S, Weltermann A, Quehenberger P, Halbmayer WM, Kyrle PA. Prediction of recurrent venous thromboembolism by the activated partial thromboplastin time. J Thromb Haemost 2006;4(04):752–756
31 Legnani C, Mattarozzi S, Cini M, Cosmi B, Favaretto E, Palareti G. Abnormally short activated partial thromboplastin time values are associated with increased risk of recurrence of venous thromboembolism after oral anticoagulation withdrawal. Br J Haematol 2006;134(02):227–232
32 Kaufmann CR, Dwyer KM, Crews JD, Dols SJ, Trask AL. Usefulness of thrombelastography in assessment of trauma patient coagulation. J Trauma 1997;42(04):716–720, discussion 720–722
33 Park MS, Martini WZ, Dubick MA, et al. Thromboelastography as a better indicator of hypercoagulable state after injury than prothrombin time or activated partial thromboplastin time. J Trauma 2009;67(02):266–275, discussion 275–276
34 Toukh M, Siemens DR, Black A, et al. Thromboelastography identifies hypercoagulablilty and predicts thromboembolic complications in patients with prostate cancer. Thromb Res 2014;133(01):88–95
35 Toh CH, Samis J, Downey C, et al. Biphasic transmittance waveform in the APTT coagulation assay is due to the formation of a Ca

(++)-dependent complex of C-reactive protein with very-low-density lipoprotein and is a novel marker of impending disseminated intravascular coagulation. Blood 2002;100(07): 2522–2529
36 Hussain N, Hodson D, Marcus R, Baglin T, Luddington R. The biphasic transmittance waveform: an early marker of sepsis in patients with neutropenia. Thromb Haemost 2008;100(01): 146–148
37 Ruberto MF, Sorbello O, Civolani A, Barcellona D, Demelia L, Marongiu F. Clot wave analysis and thromboembolic score in liver cirrhosis: two opposing phenomena. Int J Lab Hematol 2017;39(04):369–374
38 Whiting P, Al M, Westwood M, et al. Viscoelastic point-of-care testing to assist with the diagnosis, management and monitoring of haemostasis: a systematic review and cost-effectiveness analysis. Health Technol Assess 2015;19(58):1–228, v–vi
39 Harahsheh Y, Ho KM. Viscoelastic point-of-care testing to guide transfusion and antithrombotic therapy in perioperative and critically ill patients: are all parameters created equal? Anaesth Intensive Care 2016;44(01):11–13
40 Harahsheh Y, Ho KM. Use of viscoelastic tests to predict clinical thromboembolic events: a systematic review and meta-analysis. Eur J Haematol 2018;100(02):113–123
41 Francis JL, Francis DA, Gunathilagan GJ. Assessment of hypercoagulability in patients with cancer using the Sonoclot Analyzer and thromboelastography. Thromb Res 1994;74(04): 335–346
42 Akay OM, Ustuner Z, Canturk Z, Mutlu FS, Gulbas Z. Laboratory investigation of hypercoagulability in cancer patients using rotation thrombelastography. Med Oncol 2009;26(03):358–364
43 Sharma SK, Philip J, Wiley J. Thromboelastographic changes in healthy parturients and postpartum women. Anesth Analg 1997;85(01):94–98
44 Steer PL, Krantz HB. Thromboelastography and Sonoclot analysis in the healthy parturient. J Clin Anesth 1993;5(05):419–424
45 Della Rocca G, Dogareschi T, Cecconet T, et al. Coagulation assessment in normal pregnancy: thrombelastography with citrated non activated samples. Minerva Anestesiol 2012;78 (12):1357–1364
46 Shenaq SA, Saleem A. Viscoelastic measurement of clot formation: the Sonoclot. In: Ellison N, Jobes DR, eds. Effective Hemostasis in Cardiac Surgery. Philadelphia: WB Saunders; 1988:183–193
47 Peck SD. Evaluation of the in vitro detection of the hypercoagulable state using the thrombin generation test and plasma clot impedance test. Thromb Haemost 1979;42(02):764–777
48 Kelly DA, Tuddenham EG. Haemostatic problems in liver disease. Gut 1986;27(03):339–349
49 Curry NS, Davenport R, Pavord S, et al. The use of viscoelastic haemostatic assays in the management of major bleeding: a British Society for Haematology guideline. Br J Haematol 2018; 182(06):789–806
50 Davis JPE, Northup PG, Caldwell SH, Intagliata NM. Viscoelastic testing in liver disease. Ann Hepatol 2018;17(02):205–213
51 Collins PW, Macchiavello LI, Lewis SJ, et al. Global tests of haemostasis in critically ill patients with severe sepsis syndrome compared to controls. Br J Haematol 2006;135(02):220–227
52 Scarlatescu E, Juffermans NP, Thachil J. The current status of viscoelastic testing in septic coagulopathy. Thromb Res 2019; 183:146–152
53 Durila M, Lukáš P, Astraverkhava M, Beroušek J, Zábrodský M, Vymazal T. Tracheostomy in intensive care unit patients can be performed without bleeding complications in case of normal thromboelastometry results (EXTEM CT) despite increased PT-INR: a prospective pilot study. BMC Anesthesiol 2015;15:89
54 Lukas P, Durila M, Jonas J, Vymazal T. Evaluation of thromboelastometry in sepsis in correlation with bleeding during invasive procedures. Clin Appl Thromb Hemost 2018;24(06):993–997
55 Gosselin RC, Estacio EE, Song JY, Dwyre DM. Verifying the performance characteristics of the TEG5000 thromboelastogram in the clinical laboratory. Int J Lab Hematol 2016;38(02): 183–192
56 Moore HB, Moore EE, Gonzalez E, et al. Hyperfibrinolysis, physiologic fibrinolysis, and fibrinolysis shutdown: the spectrum of postinjury fibrinolysis and relevance to antifibrinolytic therapy. J Trauma Acute Care Surg 2014;77(06):811–817, discussion 817
57 Cardenas JC, Wade CE, Cotton BA, et al; PROPPR Study Group. TEG lysis shutdown represents coagulopathy in bleeding trauma patients: analysis of the PROPPR cohort. Shock 2019;51(03): 273–283
58 Adamzik M, Eggmann M, Frey UH, et al. Comparison of thromboelastometry with procalcitonin, interleukin 6, and C-reactive protein as diagnostic tests for severe sepsis in critically ill adults. Crit Care 2010;14(05):R178
59 Sivula M, Pettilä V, Niemi TT, Varpula M, Kuitunen AH. Thromboelastometry in patients with severe sepsis and disseminated intravascular coagulation. Blood Coagul Fibrinolysis 2009;20 (06):419–426
60 Peng HT, Nascimento B, Beckett A. Thromboelastography and thromboelastometry in assessment of fibrinogen deficiency and prediction for transfusion requirement: a descriptive review. BioMed Res Int 2018;2018:7020539
61 Hron G, Kollars M, Binder BR, Eichinger S, Kyrle PA. Identification of patients at low risk for recurrent venous thromboembolism by measuring thrombin generation. JAMA 2006;296(04):397–402
62 Tripodi A, Legnani C, Chantarangkul V, Cosmi B, Palareti G, Mannucci PM. High thrombin generation measured in the presence of thrombomodulin is associated with an increased risk of recurrent venous thromboembolism. J Thromb Haemost 2008;6 (08):1327–1333
63 Besser M, Baglin C, Luddington R, van Hylckama Vlieg A, Baglin T. High rate of unprovoked recurrent venous thrombosis is associated with high thrombin-generating potential in a prospective cohort study. J Thromb Haemost 2008;6(10):1720–1725
64 Faber CG, Lodder J, Kessels F, Troost J. Thrombin generation in platelet-rich plasma as a tool for the detection of hypercoagulability in young stroke patients. Pathophysiol Haemost Thromb 2003;33(01):52–58
65 Carcaillon L, Alhenc-Gelas M, Bejot Y, et al. Increased thrombin generation is associated with acute ischemic stroke but not with coronary heart disease in the elderly: the Three-City cohort study. Arterioscler Thromb Vasc Biol 2011;31(06):1445–1451
66 Alhenc-Gelas M, Canonico M, Picard V. Influence of natural SERPINC1 mutations on ex vivo thrombin generation. J Thromb Haemost 2010;8(04):845–848
67 Castoldi E, Maurissen LF, Tormene D, et al. Similar hypercoagulable state and thrombosis risk in type I and type III protein S-deficient individuals from families with mixed type I/III protein S deficiency. Haematologica 2010;95(09):1563–1571
68 Simioni P, Castoldi E, Lunghi B, Tormene D, Rosing J, Bernardi F. An underestimated combination of opposites resulting in enhanced thrombotic tendency. Blood 2005;106(07):2363–2365
69 Castoldi E, Simioni P, Tormene D, et al. Differential effects of high prothrombin levels on thrombin generation depending on the cause of the hyperprothrombinemia. J Thromb Haemost 2007;5 (05):971–979
70 Ay C, Dunkler D, Simanek R, et al. Prediction of venous thromboembolism in patients with cancer by measuring thrombin generation: results from the Vienna Cancer and Thrombosis Study. J Clin Oncol 2011;29(15):2099–2103
71 Joly B, Barbay V, Borg JY, Le Cam-Duchez V. Comparison of markers of coagulation activation and thrombin generation test in uncomplicated pregnancies. Thromb Res 2013;132(03): 386–391

72 Tripodi A, Branchi A, Chantarangkul V, et al. Hypercoagulability in patients with type 2 diabetes mellitus detected by a thrombin generation assay. J Thromb Thrombolysis 2011;31 (02):165–172

73 Debaugnies F, Azerad MA, Noubouossié D, et al. Evaluation of the procoagulant activity in the plasma of cancer patients using a thrombin generation assay. Thromb Res 2010;126(06): 531–535

74 Ollivier V, Wang J, Manly D, et al. Detection of endogenous tissue factor levels in plasma using the calibrated automated thrombogram assay. Thromb Res 2010;125(01):90–96

75 Castoldi E, Rosing J. APC resistance: biological basis and acquired influences. J Thromb Haemost 2010;8(03):445–453

76 Rühl H, Schröder L, Müller J, et al. Impact of hormone-associated resistance to activated protein C on the thrombotic potential of oral contraceptives: a prospective observational study. PLoS One 2014;9(08):e105007

77 Rühl H, Schröder L, Müller J, et al. Tamoxifen induces resistance to activated protein C. Thromb Res 2014;133(05):886–891

78 Sandset PM, Høibraaten E, Eilertsen AL, Dahm A. Mechanisms of thrombosis related to hormone therapy. Thromb Res 2009;123 (Suppl 2):S70–S73

79 Alkema L, Kantorova V, Menozzi C, Biddlecom A. National, regional, and global rates and trends in contraceptive prevalence and unmet need for family planning between 1990 and 2015: a systematic and comprehensive analysis. Lancet 2013;381 (9878):1642–1652

80 Christin-Maitre S. History of oral contraceptive drugs and their use worldwide. Best Pract Res Clin Endocrinol Metab 2013;27 (01):3–12

81 Sitruk-Ware R, Nath A. Characteristics and metabolic effects of estrogen and progestins contained in oral contraceptive pills. Best Pract Res Clin Endocrinol Metab 2013;27(01):13–24

82 Farris M, Bastianelli C, Rosato E, Brosens I, Benagiano G. Pharmacodynamics of combined estrogen-progestin oral contraceptives: 2. effects on hemostasis. Expert Rev Clin Pharmacol 2017; 10(10):1129–1144

83 European Medicines Agency. EMEA/H/A-31/1356–Assessment report for combined hormonal contraceptives containing medicinal products.Available at: https://www.ema.europa.eu/en/documents/referral/combined-hormonal-contraceptives-article-31-referral-prac-assessment-report_en.pdf. Accessed January 2020

84 Wu O, Robertson L, Langhorne P, et al. Oral contraceptives, hormone replacement therapy, thrombophilias and risk of venous thromboembolism: a systematic review. The Thrombosis: Risk and Economic Assessment of Thrombophilia Screening (TREATS) Study. Thromb Haemost 2005;94(01):17–25

85 Hotoleanu C. Genetic risk factors in venous thromboembolism. Adv Exp Med Biol 2017;906:253–272

86 Reitsma PH, Versteeg HH, Middeldorp S. Mechanistic view of risk factors for venous thromboembolism. Arterioscler Thromb Vasc Biol 2012;32(03):563–568

87 Kujovich JL. Factor V Leiden thrombophilia. Genet Med 2011;13 (01):1–16

88 Cushman M. Inherited risk factors for venous thrombosis. Hematology (Am Soc Hematol Educ Program) 2005;1:452–457

89 Kemmeren JM, Algra A, Meijers JC, et al. Effect of second- and third-generation oral contraceptives on the protein C system in the absence or presence of the factor VLeiden mutation: a randomized trial. Blood 2004;103(03):927–933

90 Mannucci PM, Franchini M. Classic thrombophilic gene variants. Thromb Haemost 2015;114(05):885–889

91 Vandenbroucke JP, Koster T, Briët E, Reitsma PH, Bertina RM, Rosendaal FR. Increased risk of venous thrombosis in oral-contraceptive users who are carriers of factor V Leiden mutation. Lancet 1994;344(8935):1453–1457

92 Vandenbroucke JP, Rosing J, Bloemenkamp KW, et al. Oral contraceptives and the risk of venous thrombosis. N Engl J Med 2001; 344(20):1527–1535

93 Lidegaard Ø, Løkkegaard E, Svendsen AL, Agger C. Hormonal contraception and risk of venous thromboembolism: national follow-up study. BMJ 2009;339:b2890

94 Westhoff CL, Pike MC, Cremers S, Eisenberger A, Thomassen S, Rosing J. Endogenous thrombin potential changes during the first cycle of oral contraceptive use. Contraception 2017;95(05): 456–463

95 Oral Contraceptive and Hemostasis Study Group. The effects of seven monophasic oral contraceptive regimens on hemostatic variables: conclusions from a large randomized multicenter study. Contraception 2003;67(03):173–185

96 Bloemenkamp KW, Rosendaal FR, Helmerhorst FM, Vandenbroucke JP. Higher risk of venous thrombosis during early use of oral contraceptives in women with inherited clotting defects. Arch Intern Med 2000;160(01):49–52

97 Hugon-Rodin J, Horellou MH, Conard J, Gompel A, Plu-Bureau G. Type of combined contraceptives, factor V Leiden mutation and risk of venous thromboembolism. Thromb Haemost 2018;118 (05):922–928

98 Henkens CM, Bom VJ, Seinen AJ, van der Meer J. Sensitivity to activated protein C; influence of oral contraceptives and sex. Thromb Haemost 1995;73(03):402–404

99 Curvers J, Thomassen MC, Nicolaes GA, et al. Acquired APC resistance and oral contraceptives: differences between two functional tests. Br J Haematol 1999;105(01):88–94

100 Alhenc-Gelas M, Plu-Bureau G, Guillonneau S, et al. Impact of progestagens on activated protein C (APC) resistance among users of oral contraceptives. J Thromb Haemost 2004;2(09): 1594–1600

101 Oslakovic S, Zadro R. Comparison of the impact of four generations of progestins on hemostatic variables. Clin Appl Thromb Hemost 2014;20(04):448–455

102 Lancé MD. A general review of major global coagulation assays: thrombelastography, thrombin generation test and clot waveform analysis. Thromb J 2015;13:1

103 Sriprasert I, Hodis HN, Bernick B, Mirkin S, Mack WJ. Association of oral estradiol dose/levels with coagulation measures in early/late postmenopausal women. Climacteric 2020;23:273–278

104 Sucker C, Tharra K, Litmathe J, Scharf RE, Zotz RB. Rotation thromboelastography (ROTEM) parameters are influenced by age, gender, and oral contraception. Perfusion 2011;26(04): 334–340

105 Tans G, van Hylckama Vlieg A, Thomassen MC, et al. Activated protein C resistance determined with a thrombin generation-based test predicts for venous thrombosis in men and women. Br J Haematol 2003;122(03):465–470

106 Tchaikovski SN, Rosing J. Mechanisms of estrogen-induced venous thromboembolism. Thromb Res 2010;126(01):5–11

107 Foidart JM, Lobo R, Rosing J, et al. Estetrol (E4) is unique native estrogen that does not modify coagulation markers in postmenopausal women and maintains sensitivity to activated protein C (APC). Paper presented at: 30th Annual Meeting of the North American Menopause Society; September 25–28, 2019; Chicago, IL

108 Ganter MT, Hofer CK. Coagulation monitoring: current techniques and clinical use of viscoelastic point-of-care coagulation devices. Anesth Analg 2008;106(05):1366–1375

109 Afshari A, Wikkelsø A, Brok J, Møller AM, Wetterslev J. Thrombelastography (TEG) or thromboelastometry (ROTEM) to monitor haemotherapy versus usual care in patients with massive transfusion. Cochrane Database Syst Rev 2011;(03):CD007871

110 Haas T, Goobie S, Spielmann N, Weiss M, Schmugge M. Improvements in patient blood management for pediatric craniosynostosis surgery using a ROTEM(®) -assisted strategy - feasibility and costs. Paediatr Anaesth 2014;24(07):774–780

111 Görlinger K, Dirkmann D, Hanke AA, et al. First-line therapy with coagulation factor concentrates combined with point-of-care coagulation testing is associated with decreased allogeneic blood transfusion in cardiovascular surgery: a retrospective, single-center cohort study. Anesthesiology 2011;115(06): 1179–1191

112 Lancé MD, Ninivaggi M, Schols SE, et al. Perioperative dilutional coagulopathy treated with fresh frozen plasma and fibrinogen concentrate: a prospective randomized intervention trial. Vox Sang 2012;103(01):25–34

113 Ay Y, Balkan C, Karapinar DY, Akin M, Bilenoglu B, Kavakli K. Feasibility of using thrombin generation assay (TGA) for monitoring bypassing agent therapy in patients with hemophilia having inhibitors. Clin Appl Thromb Hemost 2013;19(04): 389–394

114 Young G, Sørensen B, Dargaud Y, Negrier C, Brummel-Ziedins K, Key NS. Thrombin generation and whole blood viscoelastic assays in the management of hemophilia: current state of art and future perspectives. Blood 2013;121(11):1944–1950

115 Siegemund T, Petros S, Siegemund A, Scholz U, Engelmann L. Thrombin generation in severe haemophilia A and B: the endogenous thrombin potential in platelet-rich plasma. Thromb Haemost 2003;90(05):781–786

116 Al Dieri R, Peyvandi F, Santagostino E, et al. The thrombogram in rare inherited coagulation disorders: its relation to clinical bleeding. Thromb Haemost 2002;88(04):576–582

117 Nielsen VG, Cohen BM, Cohen E. Effects of coagulation factor deficiency on plasma coagulation kinetics determined via thrombelastography: critical roles of fibrinogen and factors II, VII, X and XII. Acta Anaesthesiol Scand 2005;49(02):222–231

118 Lewis SJ, Stephens E, Florou G, et al. Measurement of global haemostasis in severe haemophilia A following factor VIII infusion. Br J Haematol 2007;138(06):775–782

119 Livnat T, Martinowitz U, Zivelin A, Rima D, Kenet G. A highly sensitive thrombin generation assay for assessment of recombinant activated factor VII therapy in haemophilia patients with an inhibitor. Thromb Haemost 2011;105(04):688–695

120 Eichinger S, Lubsczyk B, Kollars M, et al. Thrombin generation in haemophilia A patients with factor VIII inhibitors after infusion of recombinant factor VIIa. Eur J Clin Invest 2009;39(08): 707–713

121 Sevenet PO, Depasse F. Clot waveform analysis: Where do we stand in 2017? Int J Lab Hematol 2017;39(06):561–568

122 Ferrante EA, Blasier KR, Givens TB, Lloyd CA, Fischer TJ, Viola F. A novel device for the evaluation of hemostatic function in critical care settings. Anesth Analg 2016;123(06):1372–1379

123 Lang T, von Depka M. Possibilities and limitations of thrombelastometry/-graphy [in German]. Hamostaseologie 2006;26(03, Suppl 1):S20–S29

124 Douxfils J, Ageno W, Samama CM, et al. Laboratory testing in patients treated with direct oral anticoagulants: a practical guide for clinicians. J Thromb Haemost 2018;16(02):209–219

125 Pernod G, Albaladejo P, Godier A, et al; Working Group on Perioperative Haemostasis. Management of major bleeding complications and emergency surgery in patients on long-term treatment with direct oral anticoagulants, thrombin or factor-Xa inhibitors: proposals of the working group on perioperative haemostasis (GIHP) - March 2013. Arch Cardiovasc Dis 2013;106(6–7):382–393

126 Levy JH, Ageno W, Chan NC, Crowther M, Verhamme P, Weitz JI; Subcommittee on Control of Anticoagulation. When and how to use antidotes for the reversal of direct oral anticoagulants: guidance from the SSC of the ISTH. J Thromb Haemost 2016; 14(03):623–627

127 Martin K, Beyer-Westendorf J, Davidson BL, Huisman MV, Sandset PM, Moll S. Use of the direct oral anticoagulants in obese patients: guidance from the SSC of the ISTH. J Thromb Haemost 2016;14(06):1308–1313

128 Seiffge DJ, Traenka C, Polymeris AA, et al. Intravenous thrombolysis in patients with stroke taking rivaroxaban using drug specific plasma levels: experience with a standard operation procedure in clinical practice. J Stroke 2017;19(03):347–355

129 Seiffge DJ, Kägi G, Michel P, et al; Novel Oral Anticoagulants in Stroke Patients study group. Rivaroxaban plasma levels in acute ischemic stroke and intracerebral hemorrhage. Ann Neurol 2018;83(03):451–459

130 Gosselin RC, Adcock DM, Bates SM, et al. International Council for Standardization in Haematology (ICSH) recommendations for laboratory measurement of direct oral anticoagulants. Thromb Haemost 2018;118(03):437–450

131 Douxfils J, Mullier F, Loosen C, Chatelain C, Chatelain B, Dogné JM. Assessment of the impact of rivaroxaban on coagulation assays: laboratory recommendations for the monitoring of rivaroxaban and review of the literature. Thromb Res 2012;130(06):956–966

132 Douxfils J, Chatelain C, Chatelain B, Dogné JM, Mullier F. Impact of apixaban on routine and specific coagulation assays: a practical laboratory guide. Thromb Haemost 2013;110(02):283–294

133 Douxfils J, Chatelain B, Chatelain C, Dogné JM, Mullier F. Edoxaban: impact on routine and specific coagulation assays. A practical laboratory guide. Thromb Haemost 2016;115(02): 368–381

134 Siriez R, Evrard J, Dogné JM, et al. Betrixaban: impact on routine and specific coagulation assays-a practical laboratory guide. Thromb Haemost 2018;118(07):1203–1214

135 Siriez R, Evrard J, Dogné JM, et al. Development of new methodologies for the chromogenic estimation of betrixaban concentrations in plasma. Int J Lab Hematol 2019;41(02):250–261

136 Hemker HC, Giesen P, AlDieri R, et al. The calibrated automated thrombogram (CAT): a universal routine test for hyper- and hypocoagulability. Pathophysiol Haemost Thromb 2002;32 (5–6):249–253

137 Marlu R, Hodaj E, Paris A, Albaladejo P, Cracowski JL, Pernod G. Effect of non-specific reversal agents on anticoagulant activity of dabigatran and rivaroxaban: a randomised crossover ex vivo study in healthy volunteers. Thromb Haemost 2012;108(02): 217–224

138 Douxfils J, Mullier F, Robert S, Chatelain C, Chatelain B, Dogné JM. Impact of dabigatran on a large panel of routine or specific coagulation assays. Laboratory recommendations for monitoring of dabigatran etexilate. Thromb Haemost 2012;107(05): 985–997

139 Herrmann R, Thom J, Wood A, Phillips M, Muhammad S, Baker R. Thrombin generation using the calibrated automated thrombinoscope to assess reversibility of dabigatran and rivaroxaban. Thromb Haemost 2014;111(05):989–995

140 Rigano J, Ng C, Nandurkar H, Ho P. Thrombin generation estimates the anticoagulation effect of direct oral anticoagulants with significant interindividual variability observed. Blood Coagul Fibrinolysis 2018;29(02):148–154

141 Bloemen S, Zwaveling S, Douxfils J, Roest M, Kremers R, Mullier F. The anticoagulant effect of dabigatran is reflected in the lag time and time-to-peak, but not in the endogenous thrombin potential or peak, of thrombin generation. Thromb Res 2018;171:160–166

142 Chowdary P, Adamidou D, Riddell A, et al. Thrombin generation assay identifies individual variability in responses to low molecular weight heparin in pregnancy: implications for anticoagulant monitoring. Br J Haematol 2015;168(05):719–727

143 Dale B, Eikelboom JW, Weitz JI, et al. Dabigatran attenuates thrombin generation to a lesser extent than warfarin: could this explain their differential effects on intracranial hemorrhage and myocardial infarction? J Thromb Thrombolysis 2013;35(02): 295–301

144 Radulovic V, Hyllner M, Ternström L, et al. Sustained heparin effect contributes to reduced plasma thrombin generation capacity early after cardiac surgery. Thromb Res 2012;130(05): 769–774

145 Hacquard M, Perrin J, Lelievre N, Vigneron C, Lecompte T. Inter-individual variability of effect of 7 low molecular weight anti-thrombin-dependent anticoagulants studied in vitro with calibrated automated thrombography. Thromb Res 2011;127(01): 29–34
146 al Dieri R, Alban S, Béguin S, Hemker HC. Thrombin generation for the control of heparin treatment, comparison with the activated partial thromboplastin time. J Thromb Haemost 2004;2(08):1395–1401
147 Robert S, Ghiotto J, Pirotte B, et al. Is thrombin generation the new rapid, reliable and relevant pharmacological tool for the development of anticoagulant drugs? Pharmacol Res 2009;59 (03):160–166
148 Bloemen S, Hemker HC, Al Dieri R. Large inter-individual variation of the pharmacodynamic effect of anticoagulant drugs on thrombin generation. Haematologica 2013;98(04):549–554
149 Sennesael AL, Larock AS, Douxfils J, et al. Rivaroxaban plasma levels in patients admitted for bleeding events: insights from a prospective study. Thromb J 2018;16:28
150 Honickel M, Braunschweig T, Rossaint R, Stoppe C, Ten Cate H, Grottke O. Reversing dabigatran anticoagulation with prothrombin complex concentrate versus idarucizumab as part of multimodal hemostatic intervention in an animal model of polytrauma. Anesthesiology 2017;127(05):852–861
151 Neal MD, Levy JH. precision correction of coagulopathy or prothrombin complex concentrates?: reversal options for dabigatran following trauma Anesthesiology 2017;127(05): 744–746
152 Dargaud Y, Wolberg AS, Luddington R, et al. Evaluation of a standardized protocol for thrombin generation measurement using the calibrated automated thrombogram: an international multicentre study. Thromb Res 2012;130(06):929–934
153 Dashkevich NM, Vuimo TA, Ovsepyan RA, et al. Effect of pre-analytical conditions on the thrombodynamics assay. Thromb Res 2014;133(03):472–476
154 Koopman K, Uyttenboogaart M, Hendriks HG, et al. Thromboelastography in patients with cerebral venous thrombosis. Thromb Res 2009;124(02):185–188
155 O'Donnell J, Riddell A, Owens D, et al. Role of the thrombelastograph as an adjunctive test in thrombophilia screening. Blood Coagul Fibrinolysis 2004;15(03):207–211
156 Woodle SA, Shibeko AM, Lee TK, Ovanesov MV. Determining the impact of instrument variation and automated software algorithms on the TGT in hemophilia and normalized plasma. Thromb Res 2013;132(03):374–380
157 Loeffen R, Kleinegris MC, Loubele ST, et al. Preanalytic variables of thrombin generation: towards a standard procedure and validation of the method. J Thromb Haemost 2012;10(12): 2544–2554
158 Perrin J, Depasse F, Lecompte T; French-speaking CAT group and under the aegis of GEHT; French-speaking CAT group (all in France unless otherwise stated); French-speaking CAT group all in France unless otherwise stated. Large external quality assessment survey on thrombin generation with CAT: further evidence for the usefulness of normalisation with an external reference plasma. Thromb Res 2015;136(01):125–130
159 Siguret V, Abdoul J, Delavenne X, et al. Rivaroxaban pharmacodynamics in healthy volunteers evaluated with thrombin generation and the active protein C system: modeling and assessing interindividual variability. J Thromb Haemost 2019;17(10): 1670–1682
160 Tripodi A. Thrombin generation assay and its application in the clinical laboratory. Clin Chem 2016;62(05):699–707
161 Rühl H, Müller J, Harbrecht U, et al. Thrombin inhibition profiles in healthy individuals and thrombophilic patients. Thromb Haemost 2012;107(05):848–853
162 Rühl H, Berens C, Winterhagen A, Müller J, Oldenburg J, Pötzsch B. Label-free kinetic studies of hemostasis-related biomarkers including D-dimer using autologous serum transfusion. PLoS One 2015;10(12):e0145012
163 Evans PA, Hawkins K, Lawrence M, et al. Rheometry and associated techniques for blood coagulation studies. Med Eng Phys 2008;30(06):671–679
164 Ovanesov MV, Ananyeva NM, Panteleev MA, Ataullakhanov FI, Saenko EL. Initiation and propagation of coagulation from tissue factor-bearing cell monolayers to plasma: initiator cells do not regulate spatial growth rate. J Thromb Haemost 2005;3(02): 321–331
165 Dashkevich NM, Ovanesov MV, Balandina AN, et al. Thrombin activity propagates in space during blood coagulation as an excitation wave. Biophys J 2012;103(10):2233–2240
166 Panteleev MA, Ovanesov MV, Kireev DA, et al. Spatial propagation and localization of blood coagulation are regulated by intrinsic and protein C pathways, respectively. Biophys J 2006; 90(05):1489–1500
167 Lipets E, Vlasova O, Urnova E, et al. Circulating contact-pathway-activating microparticles together with factors IXa and XIa induce spontaneous clotting in plasma of hematology and cardiologic patients. PLoS One 2014;9(01):e87692
168 Soshitova NP, Karamzin SS, Balandina AN, et al. Predicting prothrombotic tendencies in sepsis using spatial clot growth dynamics. Blood Coagul Fibrinolysis 2012;23(06):498–507
169 Antovic A. The overall hemostasis potential: a laboratory tool for the investigation of global hemostasis. Semin Thromb Hemost 2010;36(07):772–779
170 Simpson ML, Goldenberg NA, Jacobson LJ, Bombardier CG, Hathaway WE, Manco-Johnson MJ. Simultaneous thrombin and plasmin generation capacities in normal and abnormal states of coagulation and fibrinolysis in children and adults. Thromb Res 2011;127(04):317–323
171 Matsumoto T, Nogami K, Shima M. Simultaneous measurement of thrombin and plasmin generation to assess the interplay between coagulation and fibrinolysis. Thromb Haemost 2013; 110(04):761–768
172 Brouns SLN, van Geffen JP, Heemskerk JWM. High-throughput measurement of human platelet aggregation under flow: application in hemostasis and beyond. Platelets 2018;29(07):662–669
173 Gorog DA, Kovacs IB. Thrombotic status analyser. Measurement of platelet-rich thrombus formation and lysis in native blood. Thromb Haemost 1995;73(03):514–520
174 Suades R, Padró T, Vilahur G, Badimon L. Circulating and platelet-derived microparticles in human blood enhance thrombosis on atherosclerotic plaques. Thromb Haemost 2012;108(06):1208–1219
175 Shechter M, Merz CN, Paul-Labrador MJ, Kaul S. Blood glucose and platelet-dependent thrombosis in patients with coronary artery disease. J Am Coll Cardiol 2000;35(02):300–307
176 Shima M, Thachil J, Nair SC, Srivastava A; Scientific and Standardization Committee. Towards standardization of clot waveform analysis and recommendations for its clinical applications. J Thromb Haemost 2013;11(07):1417–1420
177 Evrard J, Morimont L, Siriez R, De Vriese M, Dogné JM, Douxfils J. Comparison of the effects of DOACs on clot waveform and thrombin generation; Special Issue: Abstracts of the XXVII Congress of the International Society on Thrombosis and Haemostasis, July 6–10, 2019. Res Pract Thromb Haemost 2019;S1:1–891
178 Dias JD, Norem K, Doorneweerd DD, Thurer RL, Popovsky MA, Omert LA. Use of thromboelastography (TEG) for detection of new oral anticoagulants. Arch Pathol Lab Med 2015;139(05):665–673
179 Seyve L, Richarme C, Polack B, Marlu R. Impact of four direct oral anticoagulants on rotational thromboelastometry (ROTEM). Int J Lab Hematol 2018;40(01):84–93
180 Artang R, Anderson M, Nielsen JD. Fully automated thromboelastograph TEG 6s to measure anticoagulant effects of direct oral anticoagulants in healthy male volunteers. Res Pract Thromb Haemost 2019;3(03):391–396

181 Müller J, Becher T, Braunstein J, et al. Profiling of active thrombin in human blood by supramolecular complexes. Angew Chem Int Ed Engl 2011;50(27):6075–6078
182 Müller J, Friedrich M, Becher T, et al. Monitoring of plasma levels of activated protein C using a clinically applicable oligonucleotide-based enzyme capture assay. J Thromb Haemost 2012;10 (03):390–398
183 Friedrich MJ, Schmolders J, Rommelspacher Y, et al. Activity pattern analysis indicates increased but balanced systemic coagulation activity in response to surgical trauma. TH Open 2018;2(04):e350–e356
184 Becher T, Müller J, Akin I, et al. The evolution of activated protein C plasma levels in septic shock and its association with mortality: a prospective observational study. J Crit Care 2018; 47:41–48
185 Rühl H, Müller J, Wäschenbach J, Oldenburg J, Dewald O, Pötzsch B. Short-term venous stasis induces fibrinolytic activation but not thrombin formation. J Atheroscler Thromb 2014;21(12):1260–1270
186 Rühl H, Winterhagen FI, Berens C, Müller J, Oldenburg J, Pötzsch B. In vivo thrombin generation and subsequent APC formation are increased in factor V Leiden carriers. Blood 2018;131(13): 1489–1492
187 Rühl H, Berens C, Winterhagen FI, et al. Increased activated protein C response rates reduce the thrombotic risk of factor V Leiden carriers but not of prothrombin 20210G>A carriers. Circ Res 2019;125(05):523–534