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

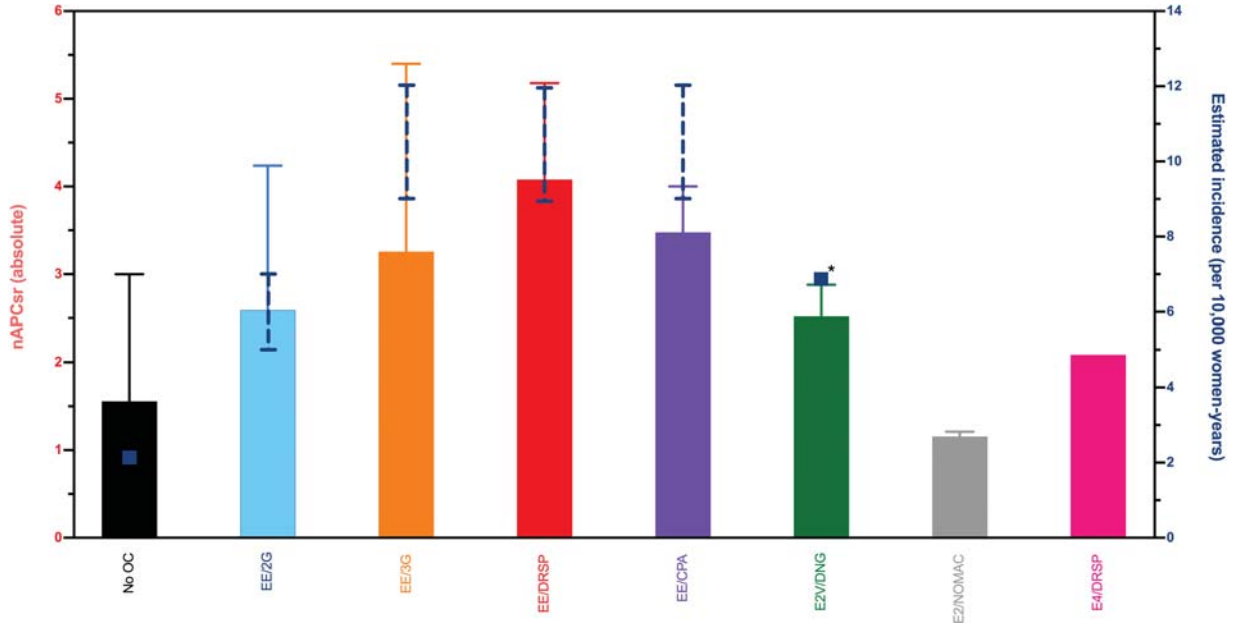

Fig. 1 Synthesis of studies from 1997 to 2019 investigating the impact of oral contraceptives on the APC resistance, when expressed as nAPCsr (absolute). Left *Y*-axis represents nAPCsr in absolute values. Right *Y*-axis represents the estimated incidence of venous thromboembolism issued from the EMA 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

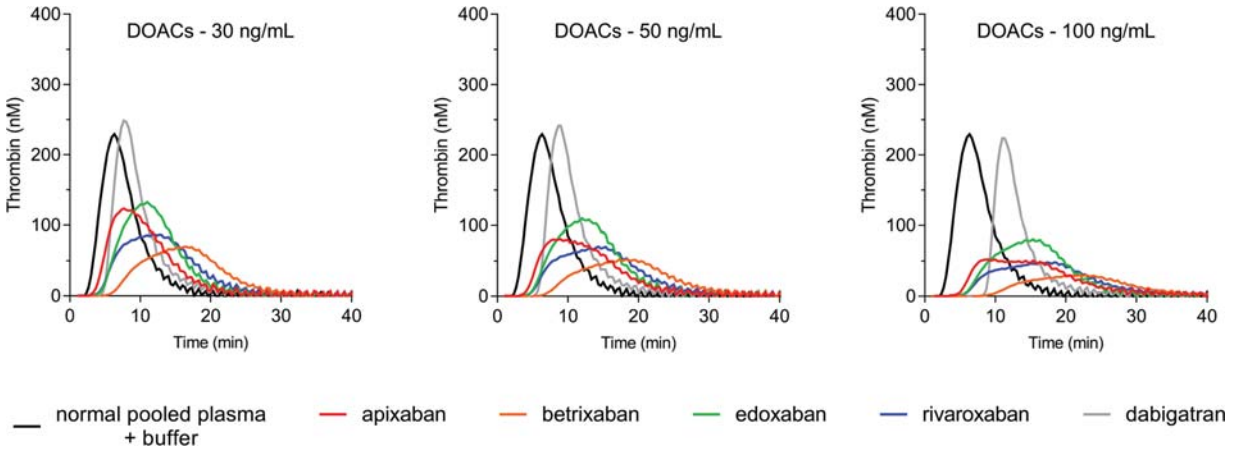

Fig. 2 Thrombin-generation profiles of different direct oral anticoagulants (DOACs) at thresholds used for antidote administration in case of bleeding or urgent surgery and to allow thrombolysis (i.e., 30, 50, or 100 ng/mL).

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.

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