Received: 2 April 2021 | Accepted: 28 September 2021

DOI: 10.1111/jth.15538

REVIEW ARTICLE

Clinical use of thrombin generation assays

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Abstract

Determining patient's coagulation profile, *i.e.* detecting a bleeding tendency or the opposite, a thrombotic risk, is crucial for clinicians in many situations. Routine coagulation assays and even more specialized tests may not allow a relevant characterization of the hemostatic balance. In contrast, thrombin generation assay (TGA) is a global assay allowing the dynamic continuous and simultaneous recording of the combined effects of both thrombin generation and thrombin inactivation. TGA thus reflects the result of procoagulant and anticoagulant activities in blood and plasma. Because of this unique feature, TGA has been widely used in a wide array of settings from both research, clinical and pharmaceutical perspectives. This includes diagnosis, prognosis, prophylaxis, and treatment of inherited and acquired bleeding and thrombotic disorders. In addition, TGA has been shown to provide relevant information for the diagnosis of coagulopathies induced by infectious diseases, comprising also disturbance of the coagulation system in COVID-19, or for the assessment of early recurrence in breast cancer. This review article aims to document most clinical applications of TGA.

KEYWORDS
coagulation assay, hemostasis, TGA, thrombin generation assay, thrombosis

1 | INTRODUCTION

Bleeding and thrombosis are challenging conditions for clinicians because of their potential critical impact on patients. Evaluating patient's hemostatic balance reflecting the risk of hemorrhage or thromboembolism is thus crucial. Routine and specialized coagulation parameters help diagnosing coagulation alterations but may fail to identify patients at bleeding or thrombosis risk. Thrombin is the pivotal enzyme of secondary hemostasis crucial to maintaining a normal hemostatic balance. Thrombin generation assay (TGA) is a global dynamic assay simultaneously and continuously measuring thrombin generation (TG) and inhibition. Because of this unique feature, TGA has been widely used as a research tool for the diagnosis, prognosis, prophylaxis and treatment of inherited and acquired coagulopathies. The availability of automated systems, offering standardized assays with common features of clinical laboratory assays could pave the way to the adoption of TGA in routine clinical practice. TGA can be run on platelet poor plasma (PPP), fresh or frozen

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Manuscript handled by: Roger Preston

Final decision: Roger Preston, 28 September 2021

platelet rich plasma (PRP) or whole blood. Despite several studies addressing pre-analytical and analytical sources of variations, TGA in the presence of platelets still lacks standardization and the precise role of platelet-dependent TG in clinical practice remains inconclusive.[1] The use of frozen PRP has been proposed as an alternative to fresh PRP as the former must be analyzed soon after blood sampling. However, comparisons of TGA results obtained with fresh and frozen PRP lead to conflicting results.[2,3] TGA run on PPP remains the most documented condition. This article provides therefore key insights into the wide array of documented applications of TGA performed in PPP, except otherwise stated, which may translate from research to clinical practice.

2 | CLINICAL AND DIAGNOSTIC APPLICATIONS IN BLEEDING DISORDERS

(most important studies are summarized in Table 1).

2.1 | Inherited hemophilia and von Willebrand disease

A challenge for clinicians is that the bleeding phenotype may vary across hemophiliac patients with the same factor level. TGA may better segregate severe hemophiliacs with identical factor levels yet distinct bleeding phenotypes according to their hemostatic capability.[5] Various supplementation therapies are available and in development based on multiple concepts (human plasma-derived and recombinant factor concentrates, extended half-life factor concentrates, bispecific antibody mimicking FVIII (FVIII), drugs targeting antithrombin, TFPI, activated protein C, or protein S (PS), gene therapy…). TGA can be used to assess how the treatment translates into increased thrombin potential protecting patients from bleeding risk [*e.g.* 6], and to help personalize hemophiliac patients' treatment. Treatment personalization using TGA was studied in surgical and non-surgical patients for factor specific replacement therapy and for non-replacement therapy (bypassing agents) or the combination thereof [*e.g.* 7]. In the former case, the initial drug pharmacokinetics was studied in a 72-hour evaluation phase including FVIII and TGA measurement, followed by a 1 to 3 months standard prophylaxis phase, and finally a personalized prophylaxis phase based on the pharmacokinetics analysis, with and TGA measured at 2, 4 and 6 months of treatment. Similarly to other studies,[8-10] the authors outline that FVIII level does not accurately reflect the coagulation capacity in hemophiliac patients. In contrast, TGA confirmed its capacity to predict serious bleeding events. In such a personalized approach, TGA, which considers the overall effect of pro- and anticoagulant proteins, may represent a useful tool for identifying patients with severe bleeding tendency who require early FVIII prophylaxis, and for optimizing and monitoring treatment efficacy. On the opposite, viscoelastic testing was considered lacking standardization for hemophilia patients' evaluation.[7] In the case of non-replacement therapy, the study conducted by Dargaud et al. showed the nature and the dose of the bypassing agent which could be advantageously chosen based on *ex vivo* experiments consisting of spiking the patient's plasma with various concentrations of the candidate drugs at and looking at which combination restored a normal TG profile. Results of the study, although conducted in a small series of patients, showed the clinical relevance of this approach, translating into the absence of bleeding in patients having received the *ex vivo* safe and efficient selected combination. Similar results were reported by Kizilocak et al. in a severe hemophiliac patient with inhibitor treated with emicizumab combined with a bypassing agent. TGA can both predict efficacy and potentially minimize the risk of thrombotic events in such patients.[11]

Development of factor-specific inhibitors is a major complication in hemophilia that exposes patients to an increased bleeding risk by decreasing factor concentrate efficacy. Factor level in combination with factor-specific inhibitor titer measured with the Nijmegen–modified Bethesda method are the reference assays to evaluate factor-specific inhibitors; however, this approach may imperfectly reflect the actual bleeding risk. As a complementary method TGA could be another indicator of the patient's coagulation status and better reflect this risk. In contrast, although being more sensitive to FVIII inhibitors, but with a narrow range of detection, thromboelastography is considered highly variable in patients with hemophilia, too unreliable for routine monitoring and to predict clinical phenotypes.[12]

Surgery in hemophiliac patients with factor-specific inhibitors is challenging and the choice of the bypassing agent is of utmost importance to improve patient outcomes and reduce costs. TGA has been proposed as an efficient tool to select the most appropriate bypassing agent and dose to ensure safe intervention.[13]

Von Willebrand disease (VWD) is the most frequent inherited bleeding disorder affecting up to 1% of the general population. However, not all patients experience significant bleeding manifestations. Beside VWD characterization, including von Willebrand

ESSENTIALS

- Evaluation of patient's hemostatic profile is crucial in many clinical situations.
- Thrombin generation assay (TGA) is a versatile global assay for the evaluation of the hemostatic balance.
- Decreased thrombin generation (TG) is associated with bleeding propensity while exaggerated TG is associated with increased thrombotic risk.
- TGA is a powerful method for diagnosis of patients with thrombotic disorders and infection-related coagulopathy.
- TGA has been shown to be an effective tool in the management of patients with inherited and acquired bleeding disorders.

TABLE 1 Summaries of most relevant TGA studies performed to assess bleeding conditions. Thrombin is a strong contributor to clot formation. Therefore, it is considered that decreased TG is associated with an increased bleeding risk, provided that the system used for measuring TG is sensitive enough. Hemorrhagic diathesis develops once TG drops below 20% of normal[4]

Clinical situation		Preferred TGA parameter	Main findings	Reference
Disease	**Objective**			
Hemophilia A with inhibitors	Monitoring bypass therapy	Peak	TGA may be a useful tool to monitor bypassing agents	6
Hemophilia A	Personalized hemophilia prophylaxis	ETP	Increased frequency of spontaneous bleeding in severe hemophilia patients with reduced ETP, irrespective of factor VIII level.	7,8,10
Hemophilia A with inhibitors	Monitoring non-factor therapy		TGA may help physicians to determine the individual profile of patients receiving combined treatment with BPA and emicizumab, and to personalize bypassing therapy when treating breakthrough bleeds.	7
Hemophilia A with inhibitors	Investigation of phenotypic heterogeneity of patients with FVIII inhibitors	ETP	ETP could be a valuable marker in monitoring patients with FVIII inhibitors.	12
Hemophilia A with inhibitors	Surgery in patients with factor inhibitors	ETP	TGT results correlate with the surgery related clinical bleeding risk and ETP may be used as a surrogate marker for monitoring bypassing agents.	13
Von Willebrand´s disease	Investigation of relationship between thrombin generation and bleeding tendency	Peak	A significant higher risk of bleeding was observed in patients with a low thrombin peak.	14
Acquired bleeding disorders	Factor inhibitors in non-hemophiliac patients	ETP	Decreased thrombin generation capacity associated with the inhibition of thrombin generation of the normal control plasma after mixing with the patient's plasma samples suggested a probable risk of bleeding.	15
Cardiovascular disease	Prediction of bleeding in cardiac surgery	ETP	TGA performed preoperatively, provides information predictive for blood loss after cardiac surgery.	17,18

factor, TGA has been proposed as an additional tool to assess the bleeding risk in VWD. Measured in PRP or PPP where, a low thrombin peak was associated with a significantly higher bleeding risk.[14]

2.2 | Acquired bleeding disorders

Development of factor-specific inhibitors outside the scope of inherited hemophilia is a rare but possibly life-threatening setting. Most of these inhibitors develop against FVIII, but other coagulation factors can also be affected. This situation generally occurs in the post-partum period, in cancer patients or autoimmune diseases, and in the elderly. Patients usually have no personal bleeding history. Laboratory diagnosis is based on routine tests and factor assays. The inhibitor titer can also be measured using the same Nijmegen-modified Bethesda method as for factor-specific inhibitors in hemophilia. The clinical impact of such inhibitors is variable and ranges from asymptomatic to a severe bleeding diathesis. TGA has been proposed to identify the effect of the factor-specific inhibitors on coagulation and possibly better identify patients at risk of bleeding. Furthermore, TGA could be an additional tool to select the most appropriate bypassing agent when required.[15]

2.3 | Prediction of bleeding in cardiac surgery

Coagulopathy after cardiopulmonary bypass (CPB) is associated with excessive blood loss and adverse patient outcomes. Thrombin plays a crucial role in primary hemostasis, and impaired TG can be an important cause of post-CPB coagulopathy. TGA can be determined

during CPB despite the high heparinization level, where it reflects the hemostatic capacity better than clotting-based assays and might better predict bleeding when performed intraoperatively.[16] Furthermore, preoperative TG is predictive for the risk of blood loss after cardiac surgery.[17-19] Conversely, in contrast to TGA, none of the parameters of viscoelastic tests correlated with the amount of bleeding in the first 24 hours.[18]

3 | THROMBOTIC DISORDERS

(most important studies are summarized in Table 2).

3.1 | Risk of deep venous thrombosis

TG was assessed very early to predict the recurrence of DVT giving a first clinical hint towards TGA being suitable for risk assessment.[22] In an ancillary study of the case – control Leiden thrombophilia study, theoretical TG profiles generated using a numerical simulation model were evaluated relative to risk of deep vein thrombosis (DVT). Results suggested that evaluating hypothetical TG based on the individual's blood composition may be useful as a predictive marker for evaluating thrombosis.[23] The ability of TGA to assess the risk of first DVT was then confirmed in another study in which an increased risk of a first venous thrombosis associated with an elevated endogenous thrombin potential (ETP) was demonstrated.[24]

3.2 | Prediction of the risk of venous thromboembolism recurrence

Patients with an inherited thrombophilia, *e.g.* defects of antithrombin, PC or PS, activated PC resistance / Factor V Leiden or the G20210A prothrombin gene mutation, carry a higher risk of thrombosis and exhibit increased TG.[25-28] After a first idiopathic venous thromboembolic event, patients are at risk of recurrence. Predisposing factors, including hereditary thrombophilia, are however weakly associated with recurrence, thus making the assessment of thrombosis recurrence challenging. On the other hand, a decision to continue or discontinue the anticoagulant treatment may expose patients to an excessive risk of bleeding or thrombosis, respectively. TGA has been proposed as a reliable tool to assess the risk of thrombosis recurrence.[22,24,29-31]

3.3 | Antiphospholipid syndrome

Antibody heterogeneity and incomplete assay standardization, particularly for lupus anticoagulants (LA), make the diagnosis of antiphospholipid syndrome (APS) problematic Furthermore, non-pathogenic aPL can be encountered in response to a variety of infections and exposure to certain medications, yet not all aPL assays can distinguish between them. The thrombotic phenotype in APS has prompted application of TGA in the research setting, revealing increased TG, often associated with acquired activated PC resistance (APC-R).[32-34]

TGA has been investigated as a potential diagnostic tool to detect on one hand the presence of aPL and on the other hand to assess the risk of thrombosis. Sheng et al. described a chromogenic assay of TG capable of detecting aPL including LA, anticardiolipin, anti-β2 glycoprotein I (aβ2GPI) and anti-prothrombin antibodies.[35] Additionally, it distinguished between aPL in APS and aPL arising from other causes. However, the assay design of using purified antibodies to inhibit TG in a normal pooled plasma (NPP) precludes it from routine diagnostic use. A multi-center study by Devreese et al. analyzed cohorts of normal donors and LA-positive patient plasmas, and a monoclonal aβ2GPI, with a typical TGA.[36] The normalized peak-height (PH)/lag-time (LT) ratio exhibited high sensitivity for LA detection, even in anticoagulated patients but further investigations are still required to distinguish from other causes of increased TG. Interestingly, subsequent work by this group proposed a method to quantify LA by using PH/LT calibration curves derived from NPP spiked with monoclonal aβ2GPI and antiprothrombin antibodies.[37] However, the *in vitro* addition of aPL to NPP resulted in TG inhibition, while APS usually exposes patients to an increased thrombotic risk. This means that TGA results in this case do not reflect APS pathophysiology in relation with thrombosis prediction.

Other studies investigated TGA as a tool to assess risk of thrombosis and recurrence in APS.[33,38-41] A study by Liestøl et al. concluded that the degree of APC-R demonstrated in their TGA in plasmas from patients with LAs was associated with thrombotic events. Zuily et al. (2013) showed that patients with aPL and TGA-demonstrated APC-R had an increased risk of incident thromboembolic events, although a history of thrombosis had a higher predictive value. Using TGA to detect aPL-induced APC-R, Arachchillage et al. found an association between high-avidity anti-PC antibodies and greater APC-R, potentially providing a marker for a severe thrombotic phenotype in APS. More recently, Zuily et al. (2020) combined detection of anti-domain I anti-β_2GPI antibodies with TGA generated activated PC sensitivity ratio as significant predictors of thrombosis over time. It seems that coupling TGA with currently employed aPL assays can provide a more sensitive and informative initial diagnostic work up. TGA can also be used to assess degree of anticoagulation of different anticoagulants in patients with APS.[42-44]

3.4 | Atherothrombosis and stroke

Coronary artery disease (CAD) is one of the leading causes of death in most wealthy countries. Increased TG is present long after acute coronary syndrome (ACS) when compared to stable patients, suggesting that patients with acute myocardial infarction generate higher, earlier, and faster thrombin, reflecting hypercoagulability.[19] As a consequence, it has been suggested that TGA could be a useful tool to guide therapy in those patients: persistence of peak thrombin

TABLE 2 Summaries of most relevant TGA studies for thrombotic conditions. Testing for individual heritable thrombophilic defects with specific component assays reflects neither gene-environment interaction nor the connectivity of the coagulation network components, *i.e.* it does not measure the composite phenotype. In this perspective, measurement of laboratory phenotypes for the stratification of thrombotic risk might be crucial. Measurement of the thrombin generating potential could provide a method for quantifying the composite effect of multiple risk factors[20,21] for thrombotic disorders which are usually related to an increased TG. Depending on the situation, different test features may have to be used

Clinical situation		Preferred TGA parameter	Main findings	Reference
Disease	**Objective**			
Thrombophilia	Assess the risk of first and recurrent venous thrombotic event by the use of TGA	ETP	Individuals with an increased ETP had an increased risk of a first deep venous thrombosis.	20
Thrombophilia	Phenotyping of venous thrombosis	ETP	TGA is sensitive to genetic variation in hemostasis-related genes, which makes it a promising tool to identify novel genetic risk factors of VTE.	21
Thrombophilia	Characterization of coagulation phenotype	Velocity index	Thrombin generation based upon the individual's blood composition is associated with the risk for thrombosis and may be useful as a predictive marker for evaluating thrombosis on an individual basis.	23
Thrombophilia	Determine the association between hypercoagulability and first and recurrent thrombosis by the use of TGA	Peak	Elevated peak was associated with an increased risk of thrombosis.	24
Thrombophilia	Investigate relationship between recurrence of VTE and TGA	Peak	Measurement of thrombin generation identifies patients at low risk for recurrent VTE.	22
Thrombophilia	Determine risk of venous thromboembolism	ETP	ETP measured in the presence of thrombomodulin may help to distinguish patients with different risk of VTE.	29
Antiphospholipid syndrome	Investigation of thrombotic events in patients with activated protein C resistance (aPC)	ETP	aPC resistance diagnosed with TGA in a majority of patients with lupus anticoagulant. A history of thrombotic events is associated with aPC resistance measured by change in ETP.	33,34
Antiphospholipid syndrome	Assess the thrombotic risk in lupus anticoagulants-positive patients	Peak /Lag time	Thrombin generation can detect the antiphospholipid syndrome with TGA as single test.	37
Atherothrombosis and stroke	Stent thrombosis	Velocity index	Stent thrombosis patients showed a hypercoagulable state detected by TGA.	47
Acute coronary syndrome	Search for predictors of cardiovascular death	ETP, Peak, Velocity index	ETP, peak thrombin and velocity index were significantly higher in patients who died compared to alive patients during 24-month follow-up.	48
Acute ischemic stroke (AIS)	Prediction of AIS	ETP	Increased thrombin generation is associated with AIS but not with coronary heart disease. Thrombin generation merges as an independent risk factor of AIS, especially in women.	49
Neonates undergoing cardiac surgery	Hypercoagulability testing and prediction of thrombosis	Peak	TGA is an independent risk factor for thrombosis and may help identify neonates at high risk for thrombosis following cardiac surgery.	50

(Continues)

TABLE 2 (Continued)

Clinical situation		Preferred TGA parameter	Main findings	Reference
Disease	Objective			
Extracorporeal membrane oxygenation (ECMO)	Coagulation phenotyping	Peak, ETP	The increase in underlying peak TG and ETP observed in adult ECMO patients ap-pears to be at least in part due to elevated Factor VIII levels, low antithrombin levels, and activated protein C resistance, which may contribute to hypercoagulability during ECMO.	

over time in acute myocardial infarction, despite antiplatelet and anti-atherosclerotic therapy, could serve as a basis for escalation to more potent antithrombotic/anticoagulant agents to prevent thrombin activation and generation in these patients.[45] Furthermore, another study suggests that the integration of blood composition data (*i.e.* fibrinogen, clotting factor levels, TFPI and antithrombin) into an assessment of TG potential can discriminate between acute and stable CAD and that a limited array of factors can be predictive.[46]

Coronary stent thrombosis is a rare, but severe, complication after percutaneous coronary intervention (PCI) with stent implantation which manifests as myocardial infarction and cardiac death. The incidence of stent thrombosis is approximately 0.5%–4%, despite optimal dual antiplatelet therapy with aspirin and an ADP-receptor antagonist. Furthermore, stent thrombosis is associated with a high recurrence rate (≈15%). Multiple factors underlie the pathophysiological mechanisms of stent thrombosis. A case–control study was conducted in 2015, including 63 patients with PCI: 23 cases (stent thrombosis) and 40 controls (no stent thrombosis). Active site-inhibited factor VIIa (ASIS) was added in one set of experiments to block TF-initiated (extrinsic pathway) coagulation and thus study the contact (intrinsic) activation system; recombinant thrombomodulin was added in another set of experiments to investigate the PC pathway. TG was significantly increased for all TF triggers when compared with controls. The addition of ASIS to the measurement without exogenous TF revealed significantly enhanced contact activation in cases compared with controls. Addition of thrombomodulin significantly reduced TG in cases *versus* controls, suggesting alterations in the PC pathway in cases. Stent thrombosis patients showed a hypercoagulable state, most likely caused by enhanced contact activation and attenuation of anticoagulation by the PC pathway.[47] These results are consistent with another study that looked at prediction of cardiovascular death in ACS patients undergoing PCI that showed despite an optimal antithrombotic therapy and a periprocedural anticoagulant treatment, a residual detectable TG in 43% of patients after PCI. These patients have a significantly higher risk of long-term cardiovascular death. Additionally, the results suggest that both residual TG and platelet reactivity are independently associated with cardiovascular death. From a clinical standpoint, TGA might help clinicians detect ACS patients at higher adverse event risk, who could benefit from a prolonged thromboprophylaxis.[48]

Acute ischemic stroke (AIS) is of major concern as stroke represents one of the main causes of mortality and disability. Identification of patients at risk may help decide the relevance of prophylaxis in selected patients. A significant association between TG and AIS has been found in an important prospective study including 9,000+ patients aged 65+ years with a 4-year follow-up period. This suggests that prevention of AIS should target the diminution of the thromboembolic risk potentially reflected by TGA.[49]

3.5 | Prediction of thrombosis consecutive to cardiac surgery in neonates

Thrombosis contributes to morbidity and mortality in neonates following cardiac surgery. TGA run preoperatively was shown to be an independent predictor of thrombosis.[50] As determined by TGA, laparoscopic sleeve gastrectomy was associated with hypercoagulability, persisting during the postoperative phase, suggesting a possible benefit of extended thromboprophylaxis.

3.6 | Extracorporeal membrane oxygenation (ECMO)

Extracorporeal membrane oxygenation (ECMO) has been proposed as an approach for achieving recovery of pulmonary function in patients with severe acute respiratory failure. In ECMO devices blood comes into constant contact with non-biological artificial materials or biomaterials leading to activation of coagulation, platelets, leucocytes and complement, which is then returned to the patient. This subsequently may lead to thrombosis, bleeding, and device failure, which remain frequent despite increasing knowledge of their pathogenesis.[51] Current laboratory tests such as prothrombin time (PT), partial thromboplastin time (aPTT), platelet count, fibrinogen, activated clotting time, or heparin dose are poor predictors of circuit thrombosis.[52] Increased D-dimer concentration is associated with the need for circuit exchange but can be unreliable as it is heavily influenced by other contributing pathologies. The TG pattern could better reflect the imbalance between procoagulant and anticoagulant factors observed in ECMO patients and may help characterize potential antithrombotic drugs.[53,54]

3.7 | Cancer-associated thrombosis (CAT)

Armand Trousseau first reported on the thrombosis – cancer relationship in 1865.

Since then, numerous studies have established thrombosis as a common complication for cancer patients, contributing to the second-leading cause of cancer patient mortality. Cancer patients are generally in a hypercoagulable or prothrombotic state, as they usually present with abnormalities in each component of Virchow's triad, stasis of blood flow, endothelial injury, and hypercoagulability, the latter including abnormalities in the coagulation and fibrinolytic pathways and platelet activation thus contributing to thrombosis.[55] The overall VTE risk is heterogeneous and dynamic over the disease course. The risk of CAT significantly increases when patient-related risk factors are present (*i.e.*, personal or family history of VTE, cardiovascular risk factors, obesity, comorbidities, etc.) or when patients are exposed to transient risk factors (*i.e.* surgery, trauma, acute infection, hospitalization, etc.). The Vienna Cancer and Thrombosis Study measured TG in 1,033 cancer patients, 77 of whom developed VTE within two years. Elevated TG, defined as PH in the 75th percentile of the study population, was found to be associated with an increased risk of VTE.[56] Lung adenocarcinoma is associated with a twofold higher VTE risk compared with other types of lung cancer, with symptomatic VTE occurring in ≈10% of ambulatory patients. In the observational ROADMAP-CAT study, the mean rate index (MRI) of the propagation phase of TG, calculated by the formula: PH/(TTP – lag-time) and expressed in nM/min, was identified as clinically relevant for the classification of ambulatory patients with lung adenocarcinoma receiving a maximum of one cycle of chemotherapy into high and intermediate/low risk for VTE.

3.8 | Breast cancer recurrence

In the HYPERCAN study, in which adult women with high-risk surgically treated breast cancer, a univariate association between cancer recurrence and ETP and PH was evidenced: higher TG levels provide a strong contribution in identifying high-risk early breast cancer recurrence patients. Furthermore, ETP could be combined with other clinical information (namely mastectomy, triple – ER/PR and HER2 – negativity and luminal B Her-2 status) in a nomogram for early recurrence risk assessment in the clinical setting.[57]

4 | APPLICATIONS TO DIAGNOSE COAGULOPATHIES IN INFECTIOUS DISEASES

4.1 | Bacterial infections and sepsis

Severe bacterial infections and sepsis are frequently associated with systemic activation of coagulation. Initiation of coagulation activation and consequent TG is caused by expression of TF on activated monocytes and endothelial cells and is ineffectually offset by TFPI. At the same time, endothelial-associated anticoagulant pathways, in particular the PC system, is impaired by pro-inflammatory cytokines. Several studies aimed to investigate the course of TG in patients with severe sepsis, and its correlation with outcome. For example, Petros et al. reported that except for ETP, there was significant difference between survivors and non-survivors of sepsis patients regarding peak thrombin, LT and time-to-peak (TTP). Peak thrombin was higher in survivors than non-survivors at all investigated time points, the LT and TTP were shorter in non-survivors than in survivors. The authors concluded that while peak thrombin showed a positive correlation with survival, the LT and TTP might be signs of impending DIC.[58] Microparticles originating from blebbing and shedding from cell membrane surfaces derived from vascular endothelium or blood cells circulate in the peripheral blood. In sepsis - especially meningococcal-induced - the genesis and role of microparticles have been well studied by TGA and other methods, and provide important insights into the underlying mechanisms of dysfunctional coagulation.[59] These studies outlined the potential TGA could have beyond classical markers of coagulation activation for a more differentiated analysis of septic coagulopathy.

4.2 | Viral infections and COVID-19

Enhanced TG in course of viral infection had been recognized before current TGA methods became available.[60] Viral infections could also be associated with reduced TG. A few cases had been described for applying TGA in differential diagnosis of viral infections [e.g. [61,62]]. With the advent of SARS-CoV-2 in humans, reports on the use of TGA for differential diagnosis and studies of virus infection related coagulopathy substantially increased.

Severe COVID-19 infection has been associated with pronounced multifactorial coagulopathy related to inflammation resulting from the cytokine storm. Excessive amounts of thrombin are generated with a subsequent hypercoagulability state leading to a high prevalence of thrombotic manifestations many with fatal outcomes. Several papers reported the use of TGA to assess the hemostatic profiles of severe COVID-19 patients. As most severe patients receive heparin at various doses, either standard or reinforced prophylactic dose, only thrombograms reflecting TGA in the presence of heparin are available. Furthermore, assay details are not always clearly documented: pre-analytical conditions, including blood sampling time *versus* heparin dosing, and analytical conditions may vary across studies and are likely to impact results. Nevertheless, TGA results confirm the excessive TG with ETP within the normal range even in the presence of heparin, and a heparin dose-dependent effect on TGA; this laboratory finding is consistent with the reduced incidence of thrombosis in patients receiving high dose heparin prophylaxis without an increase in bleeding events.[63,64] The TGA was optimized and validated to test plasma samples containing heparin, then showing a heparin dose-dependent decrease in PH and ETP. High fibrinogen did not alter the inhibitory effect of heparin, nor did it influence the PH or ETP

in any of the tested conditions.[65] In another study in 127 hospitalized patients with confirmed COVID-19, ETP and LT variables correlated with thrombo-inflammatory markers, and the D-dimer/ETP ratio could predict major adverse events during the infection.[66] Similarly, in a prospective study in 100 hospitalized COVID-19 patients thrombin peak at admission was found to be associated with an increased risk of ICU admission or death.[67]

The assumption that TGA reflects a hypercoagulable state in COVID-19 patients is however a matter of debate in the literature and some argue that test results could be due to experimental conditions and methodological issues.[68,69] In a study investigating 75 patients with critical COVID-19 as defined by WHO criteria by using a large-scale coagulation analyzer, the LT, TTP and start-tail were increased in the critical patients but there was often overlap with the noncritical group in the other TGA parameters.[70] A recent study in 46 patients with COVID-19 and 53 with sepsis among many other coagulation and fibrinolysis tests also included detailed determination of TG. In this study the influence of TF on TG parameters was investigated and diligently compared between groups of healthy controls, and patients with sepsis and COVID-19. The results suggested that interpretation of the different read-outs of TGA in the context of parameters of fibrinolysis might allow prediction of susceptibility to infection induced coagulopathies. Particularly the LT correlated positively with disease severity in COVID-19 but not in sepsis patients indicating the power of TGA in differential diagnosis.[71] In another recent small longitudinal study temporal changes in TG evaluating all read-outs normalized using a reference plasma were reported from 21 patients requiring intensive care. TGA was performed in 58% of all days with available data for measurement of laboratory parameters. In this study all patients showed LT and TTP outside the reference range. PH was elevated in 17 out of 21 patients at almost all time points over a 30 day observation period, and ETP was well above the reference range in 15 out of 21 patients, while only one patient had normal or below normal levels in all measurements.[72]

COVID-19 is still a recent disease and although extensive work has been done worldwide to better understand the pathophysiology of the disease and to improve patients' management and outcome, it is certain that more data will continue to accumulate documenting the potential contribution of TGA in this specific disease. Despite the novelty of the disease, TGA became a valuable tool to assess the overall coagulation status of patients in a relatively short time. Although COVID-19 hits the human population with the onset of a pandemic only in early 2020, several groups have used the method as a screening tool for differential diagnosis, defining severity of coagulation dysfunction, or to support therapeutic interventions.[73-75] Determination of TGA and combining with test results from different hemostatic parameters could further improve disease interpretation and therapy. Concomitant therapies, particularly treatment with anticoagulants need to be taken into consideration when interpreting results obtained by TGA, which was known before and is therefore not a specific guidance for use of TGA in COVID-19. We believe that TGA brings a different biomarker and complementary information and add to the understanding of COVID-19-associated coagulopathy, essential for further improvements in clinical care.[76] In addition to COVID-19 the rare events of unusual thrombotic events and thrombocytopenia following SARS-CoV-2 vaccination[77] have also been investigated using TGA. A first pilot study in 190 subjects vaccinated with either a vector- or an mRNA-vaccine showed no significant activation of TG.[78]

5 | ANTITHROMBOTIC THERAPY

Antithrombotic therapy including anticoagulants and antiplatelet drugs are widely used in clinical practice to prevent clot formation in various clinical settings. Regular assays measure the drug-specific activity but do not reflect the actual overall hemostatic status. As a global assay, TGA may provide a more relevant picture as described in a wide array of papers dealing with conventional drugs (vitamin K antagonists, UFH and LMWH, fondaparinux, direct Factor Xa and thrombin inhibitors).[79-86]

TGA can be a useful tool to assess effective anticoagulation in patients with venous thromboembolism.[87,88] On the other hand, as anticoagulant treatment carries a risk of bleeding, physicians may want to assess the bleeding risk in anticoagulated patients and TGA has been proposed as a useful tool in this situation too.[89]

Furthermore, no routine coagulation assay can be used to assess the reversal of anticoagulants, a situation that may be crucial in case of overdose or emergency. TGA has been proposed by several authors as a useful tool in this setting.[90,91]

6 | DISCUSSION AND CONCLUSION

Global hemostasis results from the balance between procoagulant and anticoagulant antagonisms. Conventional coagulation assays selectively explore either a specific coagulation pathway (*i.e.* the extrinsic – or TF – and common pathways for PT or the intrinsic – or contact – and common pathways for aPTT), or specific procoagulant (*e.g.* fibrinogen, FVIII, factor V) or anticoagulant (*e.g.* antithrombin, PC, PS) physiological factors. Although a pathological result may signal a bleeding propensity as in hemophilia A, or conversely may suggest an increased thrombotic risk in the case of a shortened aPTT, these assays may lack sensitivity. As such, both PT and aPTT are insensitive to physiological anticoagulant (*e.g.* antithrombin, PC, PS) defects albeit their possible contribution to an increased thrombosis risk. Moreover, the aPTT prolongation observed in the presence of a lupus anticoagulant, paradoxically does not expose the patient to an increased bleeding risk in most cases but is a proven thrombotic risk when not transitory and then part of the antiphospholipid syndrome. This emphasizes the limitations of currently used laboratory tests to assess the patient's overall hemostatic status. These limitations were to some extent, with regards to the knowledge the authors had in 1952, the rationale for proposing a TG test.[92,93]

TGA is a global assay exploring in a continuous and simultaneous manner both thrombin formation and inhibition. Thus, TGA reflects the result of pro- and anticoagulant activities and as a consequence the balanced or imbalanced hemostasis status of a patient. TGA is therefore a more representative model of *in vivo* physiology than conventional coagulation assays. Furthermore, in cases of disrupted homeostasis of hemostasis, TGA can be informative of a bleeding *versus* thrombotic tendency, which is not always the case for routine tests. Improved automation has now made TGA a more user friendly and easily accessible tool, requiring minimal technical skill, which should facilitate adoption into laboratory repertoires to complement and enhance existing assays and improve clinical outcomes.

Viscoelastic tests compete with TGA as global assays. As these offer the possibility to dynamically assess clot formation and strength in whole blood, and subsequent fibrinolysis, these assays are rather positioned as point-of-care at patient's bedside in emergency situations such as *e.g.* cardiac surgery. However, further improvement and clinical validation of viscoelastic tests remain needed.[94]

TGA still faces limitations. The first one relates to standardization. A major hindrance to wide clinical uptake of TGA was the lack of reliable commercially available standardized reagents, which prevented generation of comparable results across different clinical laboratories.[95] Recent years have seen significant efforts made by manufacturers to offer standardized reagents to facilitate comparable results in various laboratories using the same system offering commercial controlled reagents and reference material for result normalization. This resulted in a significantly reduced interlaboratory variability.[96] For further information on the technical limitations in standardization issues of TGA see *Depasse 2021*.[97] However, TGA may still benefit from standardization across different systems. One should keep in mind that, in contrast to TGA, some very common routine assays such as aPTT which are considered to give reliable readouts equally lack standardization.[98] The second main limitation relates to the lack of consensus acceptance of the use of TG for routine patient's management. Many studies, as illustrated in this review, have proven the potential of the assay, but most of them were conducted in small sets of patients and with various systems and in-house reagents. Larger studies with the use of standardized reagents and systems may certainly help a better adoption of TGA in clinical practice.

Combination of accuracy, sensitivity and versatility of up-to-date TGA-methods offers a broad spectrum of clinical and non-clinical applications. Clinical potential encompasses bleeding and thrombotic risk assessment, including diagnosis and prognosis of inherited and acquired coagulation disorders, and on their related treatment monitoring. The recent COVID-19 pandemic also revived the attention to coagulopathies related to infectious diseases, in the light of the high rate of thrombotic manifestations observed in SARS-CoV-2 infection and the need for a reinforced antithrombotic prophylaxis. TGA may contribute to the assessment of the degree of hypercoagulability in these patients and may help tailoring antithrombotic prophylaxis for a more effective and safer therapeutic regimen.

CONFLICTS OF INTEREST

Nikolaus B. Binder, François Depasse, Julia Mueller, and Thomas Wissel are employed with companies developing in vitro diagnostics including thrombin generation assays. Stephan Schwers, Matthias Germer, Björn Hermes, and Peter L. Turecek have no conflicts of interest related to contents and scope of the review manuscript.

AUTHOR CONTRIBUTIONS

Nikolaus B. Binder, François Depasse, and Peter L. Turecek designed and wrote the manuscript. Julia Mueller, Thomas Wissel, Stephan Schwers, Matthias Germer, and Björn Hermes reviewed and edited the manuscript.

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How to cite this article: Binder NB, Depasse F, Mueller J, et al. Clinical use of thrombin generation assays. *J Thromb Haemost*. 2021;19:2918–2929. doi:10.1111/jth.15538