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Review Article

The current status of viscoelastic testing in septic coagulopathy☆

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ABSTRACT

Sepsis can be associated with different degrees of coagulopathy, ranging from a mild activation of the coagulation system to disseminated intravascular coagulation (DIC). The evaluation of haemostasis in the context of sepsis is important since it has been shown that anticoagulant therapies were beneficial mainly in patients with sepsis-induced DIC, but not in the general population of septic patients. Sepsis-induced haemostatic disturbances are not adequately reflected by standard coagulation tests (SCTs) which only consider the plasmatic components of the haemostatic system and not the cellular components. In addition, SCTs only assess the initiation phase of coagulation and reflect the activity of pro-coagulant factors, but lack sensitivity for the anticoagulant drive and the fibrinolytic activity. Viscoelastic tests (VET) are whole-blood tests which can assess clot formation and dissociation, and the contribution of both plasmatic and cellular components with a shorter turnaround time compared to SCTs. The use of VET in septic patients has proved useful for the assessment of the fibrinolytic activity, detecting hypercoagulable status and for the diagnosis of DIC and mortality risk prediction. While having relevant advantages over SCTs, the VET also present some blind spots or limitations leaving space for future improvement by the development of new reagents or new viscoelastic parameters.

1. Introduction

Sepsis is usually associated with different degrees of coagulopathy, ranging from a mild activation of the coagulation system to the more severe end of the spectrum with overt disseminated intravascular coagulation (DIC) [1–3]. The main pathophysiological mechanisms of sepsis-associated coagulopathy are represented by the increased expression of tissue factor on the surface of circulating endothelial cells and by the impairment of the anticoagulant and fibrinolytic pathways [2]. This state of increased coagulant activity and decreased fibrinolysis induced by inflammation leads to fibrin deposition in the microcirculation which contributes to organ dysfunction [1,2]. Both organ dysfunction and mortality are increased in septic patients with severe coagulopathy and/or DIC [4].

Based on the pathophysiological changes in sepsis, one would expect a beneficial effect of anticoagulant therapies. However, many large trials testing different anticoagulants in septic patients showed heterogenous results [5–9]. According to Umemura et al., a beneficial effect of anticoagulant therapy on mortality was observed only in the subpopulation of sepsis-induced DIC but not in the overall sepsis population [10]. Currently, the anticoagulant therapy is not recommended by the Surviving Sepsis Campaign Guidelines 2016 while its use is suggested by the Japanese guidelines for selected patients with sepsis-induced DIC [11–13].

As the coagulation system plays a major role in the pathophysiology of multiple organ dysfunction syndrome in sepsis, the assessment of haemostasis is mandatory for selecting the adequate patient population for interventions targeting the coagulation system in sepsis and is also important for patient outcome prediction and for the development of new treatment strategies.

The coagulation system has been historically assessed by standard coagulation tests (SCTs) such as prothrombin time (PT) and activated partial thromboplastin time (aPTT). PT and aPTT are measured as the time (in seconds) necessary for plasma to clot after the addition of calcium and an activator of the extrinsic and, respectively, the intrinsic pathway [14]. These tests mainly reflect the activity of pro-coagulant factors in the circulation without sensitivity for the anticoagulant drive which occur simultaneously in the septic patients. In addition, the SCTs only take into account the plasmatic components of the haemostatic system while the important contribution of the cellular components including platelets and red cells is neglected.

Rotational thromboelastometry (ROTEM) and thrombelastography

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Table 1
TEG® and ROTEM® parameters and their significance.

ROTEM parameter	TEG parameter	Definition	Main influencing factors	Significance
CT (clotting time) in s	R (reaction time) in min	Time to 2 mm clot firmness amplitude	Coagulation factors, anticoagulants, tissue factor expression	Clot initiation
CFT (clot formation time) in s	K (kinetic time) in min	Time from 2 mm to 20 mm clot firmness amplitude	Thrombin generation, platelets, fibrinogen	Clot kinetics
α angle in degrees	α angle in degrees	Angle between the tangent to the clotting curve through the 2 mm point and the horizontal midline		
MCF (maximum clot firmness) in mm	MA (maximum amplitude) in mm	The maximum amplitude reached during the test.	Platelets, fibrinogen, factor XIII	Clot strength
LI30, 45, 60 (lysis index at 30, 45, 60 min after CT) in % of MCF	LY30, 60 (lysis index at 30, 60 min after MA) in % of MA	LI30, 45, 60 = residual clot firmness at 30, 45, 60 min after CT (ROTEM). LY30, 60 = the amount of lysis at 30, 60 min after MA (TEG)	Fibrinolytic enzymes, fibrinolytic inhibitors, factor XIII	Clot lysis

(TEG) are whole-blood point-of-care tests (also called viscoelastic tests or VET) increasingly used in the bedside management of bleeding during liver transplant, cardiac surgery and in trauma patients [15].

1.1. Brief description of TEG and ROTEM analysis

These tests assess the physical proprieties of the blood clot formed in a cup with a pin suspended inside the blood [16]. In TEG technology, there is a movement of the cup and the pin is fixed, while in ROTEM the cup is fixed and the pin is moving [15]. During the clotting process an increasing number of fibrin strands are formed between pin and cup-wall [17]. In this way, the relative movement of the cup and pin is directly related to the strength of the formed clot. The amplitude of this movement is recorded by the VET devices and, using dedicated software, is transformed in ROTEM or TEG parameters [17]. The most important parameters provided by both viscoelastic devices are presented in Table 1 [15,17] and in Fig. 1.

VET are able to assess clot formation, stabilization and lysis, revealing the contribution of both plasmatic and cellular components [15]. Even if VET are closer to the “in vivo” conditions compared to SCTs, they fail to reflect the important contribution of other elements found “in vivo” such as the blood flow or the endothelium. The results of viscoelastic tests are influenced by various preanalytical factors [18]; the result ranges can be also modified in patients with extreme haematocrit levels or at high altitude compared with sea level [19,20].

From a practical point of view, the use of an additional diagnostic test is justified if the results would bring new information relevant for the patient's treatment or outcome prediction. The use of VET in septic patients might be useful to uncover some blind spots in the assessment of septic coagulopathy performed by SCTs such as i) the quantification of fibrinolysis activity, ii) the diagnosis of hypercoagulability, iii) the prediction of DIC with a shorter turnaround time. This review underlines the strong and weak points of VET in critically-ill septic patients emphasizing their usefulness in daily clinical practice in conjunction with SCTs.

2. Viscoelastic tests in the diagnosis and quantification of fibrinolysis inhibition in sepsis

2.1. The problems with current tests in identifying fibrinolysis activation and inhibition

Together with coagulation activation, the fibrinolytic system is activated in order to remove the clots from the vascular system and restore the flow through vessels. This is achieved after the conversion of the inactive proenzyme plasminogen to the active enzyme plasmin which degrades fibrin into soluble degradation products [21]. Plasmin formation mainly occurs after the assembly of the plasminogen-tissue plasminogen activator (t-PA) complex on the surface of fibrin and plasminogen cleavage [21]. Similar to coagulation, fibrinolysis is regulated by different activators and inhibitors.

Activation of the fibrinolytic system with plasmin formation is possible by two pathways: by tissue-type plasminogen activator (t-PA) or by urokinase-type plasminogen activator (u-PA), both inhibited by plasminogen activator inhibitors (PAI-1 and PAI-2) [22]. In vivo, the activity of plasmin is rapidly inhibited by antiplasmin, by the formation of stable complexes (plasmin-antiplasmin complexes, PAP) [22]. The activation of plasmin and its inhibitors can happen to varying extents at different stages of haemostatic process. In sepsis specifically, the fibrinolytic activity depends on the balance between t-PA and PAI-1. However, various other factors such as PAI-2, thrombin-activatable fibrinolysis inhibitor (TAFI), increased plasma levels of nuclear products such as cell-free DNA and histones, neutrophil elastases and different proteases which all influence fibrinolysis, can complicate the assessment of fibrinolysis [23,24]. By releasing different regulators of the fibrinolytic process during different stages, the endothelium is

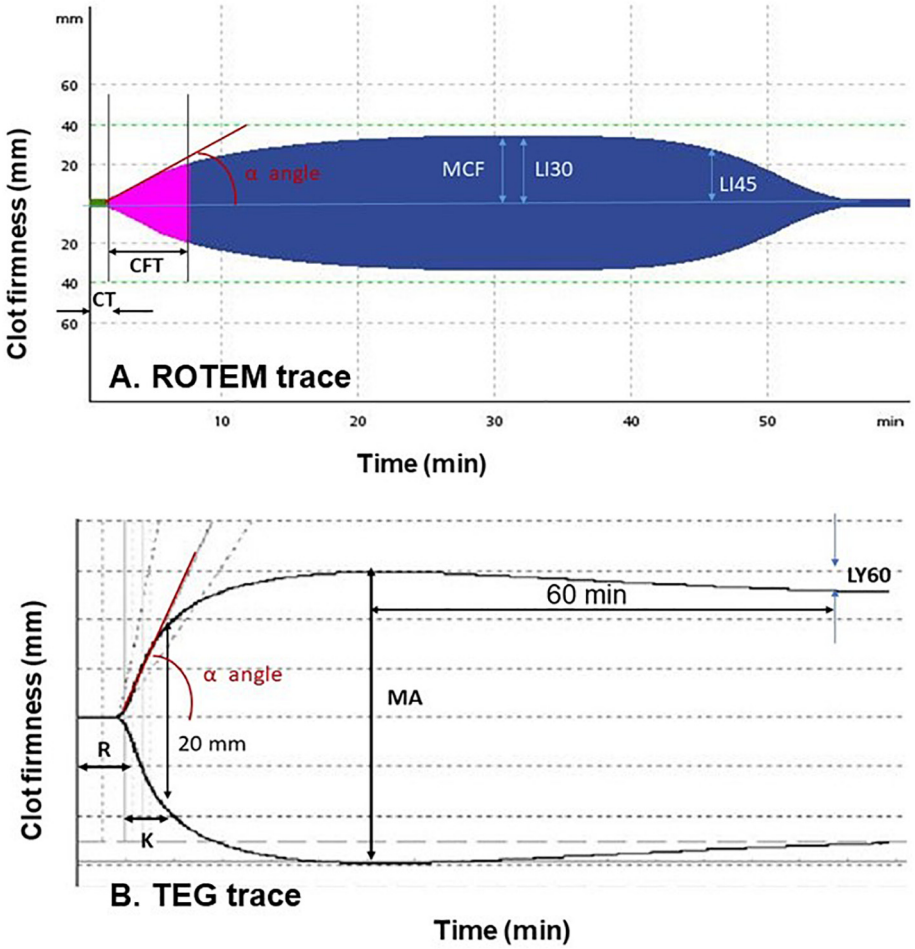

Fig. 1. A. ROTEM trace; B. TEG trace.
CT = clotting time, CFT = clot formation time, MCF = maximum clot firmness, LI30,45 = lysis index at 30, 45 min after CT.
R = reaction time, K = kinetic time, MA = maximum amplitude, LY60 = lysis index at 60 min after MA.

responsible for the initial pro-fibrinolytic response seen in early inflammation and for the fibrinolysis inhibition observed in later stages of sepsis [25–27].

This early fibrinolytic activation has been captured in experimental studies on healthy volunteers after triggering an inflammatory response by the administration of low doses of endotoxin or tumour necrosis factor, while clinical research involving septic patients only described the later stage of fibrinolytic shutdown revealed by the measurement of increased levels of fibrinolytic inhibitors or by global tests such as euglobulin clot lysis time or VET [25,27–31]. Despite the recognized role of fibrinolysis inhibition as a contributor to the development of organ dysfunction in sepsis and despite the numerous studies testing treatments that target sepsis inflammation or coagulopathy, a specific measure designed to correct the low fibrinolytic activity has not yet been assessed in large trials [23]. The research on the potentiation of fibrinolytic activity in sepsis by different interventions is sparse [32–36]. This is probably due to the difficulties associated with the diagnosis and quantification of fibrinolytic activity which is not reflected by routine coagulation tests, but rather by special techniques that are cumbersome and time consuming [37].

Fibrinolysis can be estimated based on the measurement of individual fibrinolytic components and on global tests of fibrinolysis. The interpretation of elevated levels of fibrinolysis-related biomarkers is extremely complicated in states with increased coagulation activation such as sepsis or septic shock. The plasmatic levels of circulating D-dimers and fibrin degradation products are dependent on both coagulation and fibrinolysis activation [38]. Mavrommatis et al. found markedly elevated markers of coagulation (thrombin-antithrombin complexes) and of fibrinolytic activation (D-dimers, plasmin-antiplasmin complexes) in septic patients compared to healthy controls, in contrast with the fibrinolytic activity inhibition demonstrated using global haemostatic or fibrinolysis tests in such patients [29,30,39,40]. The fibrinolysis-related biomarkers such as D-dimers and plasmin-antiplasmin complexes (PAP) reveal an ongoing or a recent fibrinolytic activity, but this can vary depending also on their plasmatic clearance.

Global tests of fibrinolysis performed using plasma or the euglobulin fraction of plasma are in general cumbersome, time consuming and available only in specialized laboratories [37]. Euglobulin clot lysis time is one of the tests used for fibrinolysis assessment using the euglobulin fraction of plasma which is obtained by acidification of platelet-poor plasma resulting in a significant depletion of fibrinolysis inhibitors with relatively preserved levels of plasminogen activators [37,41]. Other tests used for global fibrinolysis assessment in whole blood are the viscoelastic tests where clot lysis is estimated by the decrease in clot amplitude related to the maximum amplitude reached during measurement [41]. However, one should take into account that fibrinolytic activity as assessed by VET will not reflect all the regulatory factors of fibrinolysis as the endothelial contribution to fibrinolysis is neglected.

2.2. The usefulness of viscoelastic tests in identifying fibrinolysis activation and inhibition

Using VET different fibrinolytic phenotypes were described in

trauma patients and correlated with outcome, with the highest rates of mortality observed in patients with both fibrinolysis shutdown and hyperfibrinolysis [42]. Albeit not rare, fibrinolysis shutdown is less acknowledged compared to hyperfibrinolysis in trauma patients, probably due to late clinical manifestations in the form of organ dysfunction and it is mainly due to elevated levels of PAI-1 activity released from platelets or endothelium [43].

Some studies on trauma patients suggested that VET have a low sensitivity for the detection of hyperfibrinolysis [44,45]. In their study, Raza et al. diagnosed hyperfibrinolysis using ROTEM only in patients with markedly elevated PAP complexes reaching 30 times the normal value, while an increased clot lysis was not detected in patients with a moderate increase in PAP levels [44]. In a recent study, Cardenas et al. included trauma patients from the PROPPR cohort and demonstrated that fibrinolysis shutdown diagnosed by decreased clot lysis on TEG correlated with moderate or high PAP levels [45].

Fibrinolysis inhibition is even more difficult to diagnose using VET, because at baseline, normal healthy individuals demonstrate only minor clot lysis [18]. The use of triggers to activate the coagulation with TEG or ROTEM is associated with a lower sensitivity for hyperfibrinolysis detection. It is possible that using less-powerful activators or even non-activated samples allows a better fibrinolytic activity detection [46,47]. As a consequence, the absence of clot lysis on ROTEM or TEG result can stand for (undetected) hyperfibrinolysis, normal fibrinolytic activity or fibrinolytic shutdown and one should carefully consider this aspect when interpreting the fibrinolytic activity based on VET. In studies using global haemostatic assays such as ROTEM or TEG, decreased clot lysis indices were found in patients with sepsis and septic shock compared to healthy controls [30,40,48]. In some studies, VET were modified by adding fibrinolysis activators in order to increase sensitivity for the detection of changes in the fibrinolytic system [29,30]. A hypofibrinolytic profile was revealed in septic patients after the addition of t-PA or u-PA in the samples [29,30]. However, despite the low clot lysis observed with non-modified VET assays, Panigada et al. described two populations of septic patients: normal and low responders, based on the clot lysis produced on VET by adding fibrinolysis activators [30]. In this way, a real fibrinolysis shutdown was demonstrated using the modified TEG only for the low-responders group of patients which also had increased ICU mortality compared to the normal responders group [30]. It is difficult to identify septic patients with impaired fibrinolysis without using modified VET, as septic patients with normal and with low response to fibrinolysis activators are very similar when assessed using plasmatic markers of fibrinolytic activity or standard VET [30].

2.3. Possible directions for improving fibrinolysis assessment using VET

Lysis indices reflecting fibrinolysis are non-parametric measurements based on the changes in amplitude after the maximum clot firmness is reached. However, as clot build-up and fibrinolysis overlap in time, fibrinolytic activity affects different aspects such as clot firmness and kinetics and is also reflected by the kinetics of clot formation before reaching the point of maximal amplitude [49]. The use of velocity curves in comparison with lysis indices could increase the sensitivity of VET for the detection and assessment of fibrinolysis, thus allowing the earlier detection of hyperfibrinolysis [50–52]. There is still room for improvement in the diagnosis and quantification of fibrinolysis inhibition in septic patients using ROTEM or TEG. For this matter, the use of VET seems very promising and the development of new reagents or of new parameters might allow in the near future the identification of septic patients with fibrinolytic shutdown that could benefit from targeted therapies [53].

3. Viscoelastic testing for hypercoagulability detection in sepsis

3.1. The usefulness of viscoelastic tests for the assessment of hypercoagulability in critically ill septic patients

One of the advantages of VET over SCTs is their capacity to detect hypercoagulability. It was demonstrated that ROTEM or TEG could be useful to diagnose hypercoagulability and even to predict the development of thrombotic complications in special patient populations, such as postoperative or prostate cancer patients [54–57].

Septic patients often have reduced levels of coagulation factors, usually not below the haemostatic threshold, but still leading to the prolongation of SCTs suggesting hypocoagulability; however, by using more global haemostatic tests, a different coagulation profile may be revealed [58,59]. Using thrombin generation tests, Collins et al. demonstrated a similar amount of thrombin generated in sepsis patients compared to controls, after a delayed initiation of coagulation [58]. The use of ROTEM resulted in similar findings as the thrombin generation assay, with septic patients demonstrating normal or exaggerated clot formation after a delayed initiation phase (prolonged clotting time while the alpha angle and clot firmness were increased) [58]. Similar results of abnormally prolonged SCTs with normal or hypercoagulable VET results were also reported in cancer patients with sepsis or septic shock [60]. Previous publications show that in critically ill septic patients with prolonged PT but normal or hypercoagulable ROTEM results, invasive procedures could be performed without bleeding complications [61,62].

Other studies in patients with sepsis or septic shock reported prolonged PT or aPTT, delayed and decreased thrombin generation suggesting hypo-coagulability while thromboelastometric parameters generally remained within reference interval range [63,64]. A possible explanation of this result is that VET measure primarily clot amplitude-based parameters, making possible for increased fibrinogen levels to mask other mild or moderate haemostatic factors deficiencies which are mirrored by SCTs [64].

A significant increase of the clot rate formation and firmness was described in patients with sepsis and severe sepsis compared to healthy controls, consistent with hypercoagulability which was significantly diminished in septic shock patients [65]. The earlier stages of sepsis represent a hypercoagulable phase which progresses towards a hypocoagulability in more advanced stages of sepsis, but these coagulation system changes are not revealed by SCTs [64,65].

3.2. Possible directions for improving haemostasis assessment in sepsis patients using VET

In sepsis, the plasmatic levels of coagulation factors and inhibitors are decreased, with the exception of factor VIII and fibrinogen. However, the VET might not be sensitive to changes in anticoagulant factor levels, as demonstrated by Collins et al. for the low-tissue factor activated ROTEM, possibly resulting in the underestimation of hypercoagulability in septic patients [58]. In an in-vitro study using TEG, Nielsen et al. demonstrated that the use of activated assays resulted in a lower sensitivity for antithrombin deficiency detection compared to non-activated assays, due to the increased and faster thrombin generation overwhelming the inhibitory effects of antithrombin [66]. Whole-blood VET include the effect of the cellular component on blood coagulation, but due to the lack of endothelial cells, the natural anticoagulant pathways are not activated and are not adequately reflected by VET testing. In this way, the balance between the procoagulant and anticoagulant drives is not adequately reflected by tests where the full activation of the protein C is not allowed [67]. Thrombomodulin, a glycoprotein localized on the surface of endothelial cells, binds thrombin forming a complex which is a potent activator of both Protein C (PC) and thrombin-activatable fibrinolysis inhibitor (TAFI) [68]. PC and TAFI get activated after binding to different domains of the

thrombin-thrombomodulin complex and their activation results in different actions: activated PC has anticoagulant and pro-fibrinolytic effects while activated TAFI attenuates fibrinolysis [68].

A modified thrombin generation test with thrombomodulin addition for a better detection of plasmatic anticoagulant activity was described by Tripodi et al., which demonstrated in their study a rebalanced haemostatic system in cirrhotic patients where the deficiency of pro-coagulant factors is balanced by a concomitant decrease in anticoagulants [67]. A similar concept might also be used with whole blood VET. A novel test incorporating endothelial cells in the ROTEM cup revealed a pro-coagulant effect with decreased clotting time compared with the non-modified test, explained by the increased expression of endothelium-derived TF, a result opposite to the expectation that the endothelium would have an anti-coagulant effect [69]. A recent study demonstrated that the addition of thrombomodulin and activated protein C to whole blood tissue-factor activated TEG samples resulted in the prolongation of TEG parameters with a more important effect of activated protein C compared to thrombomodulin [70]. More research is needed for the development of standardized reagents and tests allowing the in vitro activation of anticoagulant systems for a better assessment of the pro and anti-coagulant balance with whole-blood VET.

4. Viscoelastic testing for the early diagnosis and/or prediction of DIC

The diagnosis of overt DIC in sepsis is based on different scoring systems such as ISTH (International Society for Haemostasis and Thrombosis) DIC score or JAAM (Japanese Association for Acute Medicine) DIC score calculated using SCTs, platelet count and the assessment of the plasmatic levels of fibrin degradation products or D-dimers [4,71]. In 2001, the ISTH proposed a scoring system for the diagnosis of non-overt DIC taking into account serial changes over time of the coagulation status assessed by SCTs results, antithrombin and protein C levels [72].

Majority of sepsis patients are not identified in the early phase of hypercoagulability (non-overt DIC), but rather when the sepsis-induced haemostatic dysfunction is decompensated and already progressed to overt DIC. However, in the early phase of non-overt DIC, the VET are able to reflect the activation of coagulation leading to hypercoagulability which is not detected by standard coagulation tests [73]. In an experimental study by Schochl et al., the early activation of coagulation induced by intravenous endotoxin administration in animals was detected by ROTEM, but not by SCTs [73]. Using ROTEM, Adamik et al. identified septic patients with hyper, hypo or normo-coagulable pattern at ICU admission [74]. The presence of coagulation disorders (both hyper and hypocoagulability) on ROTEM correlated with a significantly higher mortality rate compared to normocoagulable patients while SCTs were not able to detect the group of septic patients with hypercoagulability having also increased mortality risk [74].

In their group of sepsis patients, Sivula et al. showed that ROTEM differentiated between septic patients with or without overt DIC, by revealing hypocoagulability in patients with overt DIC and a trend towards hypercoagulation in those without overt DIC as compared with healthy controls [48]. In this way, the use of VET differentiates between the hypo and hypercoagulable profiles of critically ill septic patients having prolonged SCTs, which is extremely important in clinical practice for limiting the pro-coagulant interventions in septic patients with hypercoagulable state despite their abnormal SCTs [48]. In their study, Sharma et al. also found disturbed TEG parameters suggesting hypocoagulability in septic patients with overt DIC and by using a score combining four TEG parameters the sensitivity and specificity for DIC prediction increased [75]. In a small sample of septic patients, it was demonstrated that clotting time (CT) with tissue-factor triggered ROTEM was strongly correlated with JAAM DIC score and had very good specificity and sensitivity for DIC prediction, similar to PT, INR and plasma concentration of fibrin degradation products [76].

Sepsis can be associated with coagulation disturbances of different severity and with variable patterns which cannot be identified by SCTs alone; the use of VET might provide a more detailed view, and reveal simplified and targeted interventions for the optimization of the coagulopathy. In sepsis, overt DIC is associated with hypocoagulability revealed by VET parameters such as prolonged CT/R and CFT/K and by decreased clot amplitude [48,75,77]. The use of VET provides a useful orientation towards the diagnosis of DIC including non-overt DIC without the delays usually associated with laboratory measurements. Many trials of anticoagulant treatments in sepsis were not successful due to inadequate selection of patients and/or to the delaying too much the drug administration. In this note, VET could also prove useful for the timely identification of patients with overt DIC and for the appropriate selection of target patients for inclusion in trials of anticoagulant therapies in sepsis.

5. Blind spots of viscoelastic testing in sepsis

In sepsis patients, the VET are useful to reveal plasmatic coagulation disturbances which are correlated with worse prognosis, however these tests are unable to detect haemostatic changes occurring in sepsis which are also associated with increased mortality such as platelet dysfunction or increased von Willebrand factor levels. It is well-known that thrombocytopenia is correlated with increased mortality in patients with sepsis and septic shock [78–80]. In their cohort of septic patients, Brenner et al. revealed decreased platelet aggregation in response to both weak and strong agonists in septic patients compared to controls [77]. The reduced platelet aggregability was even more important in septic patients with overt DIC compared to patients without DIC [77]. Impaired platelet aggregation was found to be correlated with increasing sepsis severity and with poor outcome [81,82]. In sepsis, both platelet count and function may serve as valuable prognosis tools for outcome prediction [81,82]. VET are useful to detect low platelet counts, however they are insensitive for detecting platelet function defects due to the strong platelet activation by thrombin [17].

Another “blind spot” of the VET is the inability to assess von Willebrand factor (VWF) quantity and activity. In sepsis, due to increased inflammation and subsequent endothelial activation, the increased release of (ultra-large) VWF multimers is observed in conjunction with the depletion of the enzyme responsible for their cleavage, ADAMTS-13, leading to thrombotic microangiopathy, also known as a TTP (thrombotic thrombocytopenic purpura)-like syndrome [83,84]. This imbalance between the levels of VWF and ADAMTS-13 is correlated with sepsis severity and outcome [85–87]. However, these prognostic factors and the development of thrombotic microangiopathy features in sepsis patients are not reflected by VET.

6. Conclusions

VET reveal decreased clot lysis in septic patients. They represent a very convenient method for clot lysis assessment, despite their limited sensitivity for the quantification of fibrinolytic activity. As opposite to SCTs, the VET are useful for the detection of hypercoagulability often found in the early stages of sepsis, allowing the identification of patients at risk for developing thrombotic complications despite having prolonged SCTs. By revealing the hypocoagulable status found in later stages of sepsis, the VET are useful for the rapid identification of sepsis-induced overt DIC and for outcome prediction in sepsis patients, as the development of hypocoagulability is strongly correlated with increased mortality. However, the VET are not suitable to detect other haemostatic alterations also correlated with worse outcome, such as platelet dysfunction or ADAMTS-13 deficiency and ultra-large VWF multimers.

More research is needed for the development of new reagents and/or tests allowing the in vitro activation of anticoagulant systems for a better assessment of the pro and anti-coagulant balance with whole-blood VET. This would allow for a better assessment of fibrinolytic

activity and should be regarded as a high priority for the future development of VET.

Declaration of competing interest

None.

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