

Utility of Global Hemostatic Assays in Patients with Bleeding Disorders of Unknown Cause

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Abstract

Bleeding disorder of unknown cause (BDUC) is a diagnosis of exclusion after exhaustive evaluation of plasmatic coagulation and platelet function. This review explores the utility of global hemostatic assays as confirmatory tests and in elucidating the pathophysiology of BDUC. Unlike traditional hemostatic tests that focus on coagulation factors, global assays are conducted both in plasma and also whole blood. These assays provide a more comprehensive understanding of the cell-based model of coagulation, aid in the identification of plasmatic factor abnormalities that may reduce hemostatic capacity, and allow for the assessment of impaired platelet–endothelial interactions under shear stress, as well as hyperfibrinolytic states. While clinical tests such as skin bleeding time and global assays such as PFA-100 exhibit limited diagnostic capacity, the role of viscoelastic testing in identifying hemostatic dysfunction in patients with BDUC remains unclear. Thrombin generation assays have shown variable results in BDUC patients; some studies demonstrate differences compared with healthy controls or reference values, whereas others question its clinical utility. Fibrinolysis assessment in vitro remains challenging, with studies employing euglobulin clot lysis time, plasma clot lysis time, and fluorogenic plasmin generation yielding inconclusive or conflicting results. Notably, recent studies suggest that microfluidic analysis unveils shear-dependent platelet function defects in BDUC patients, undetected by conventional platelet function assays. Overall, global assays might be helpful for exploring underlying hemostatic impairments, when conventional hemostatic laboratory tests yield no results. However, due to limited data and/or discrepant results, further research is needed to evaluate the utility of global assays as screening tools.

Keywords

- ▶ global assays
- ▶ von Willebrand disease
- ▶ platelet function defects
- ▶ hemophilia A/B
- ▶ BDUC

Introduction

Individuals with mild to moderate bleeding disorders (MBDs) typically exhibit various symptoms including mucocutaneous bleeding, postsurgical bleeding, or postpartum hemorrhage (PPH).¹ For a significant majority of these patients, the cause of their bleeding disorder remains elusive.^{1,2} Bleeding disorder of

unknown cause (BDUC) is defined as patients with a nontrivial mild-to-moderate bleeding tendency with normal results on complete blood count; prothrombin time (PT); activated partial thromboplastin time (APTT); thrombin time (TT); von Willebrand factor (VWF) antigen and activity; coagulation factors VIII, IX, and XI; and platelet light transmission aggregometry.³ In addition, acquired and non-hemostatic causes of

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bleeding such as hematological disorders (e.g., amyloidosis), hereditary hemorrhagic telangiectasia, endocrinological conditions (e.g., hypothyroidism), or scurvy must be excluded before diagnosing patients with BDUC.^{4,5} These adult patients with BDUC share a remarkably similar bleeding profile, both in terms of symptoms and severity, with other MBD diagnoses such as von Willebrand disease (VWD) types 1 and 2, platelet function defects (PFDs), or non-severe coagulation factor deficiencies (CFDs) that are not diagnosed until childhood.^{5–7}

Patients with BDUC have gained recognition over the past decade,⁸ primarily due to the significant disease burden that affects both their physical and mental well-being.⁹ As BDUC is a “diagnosis of exclusion,” the extensive diagnostic workup of plasmatic coagulation and platelet function is not always feasible and usually restricted to specialized tertiary centers.^{10,11} Currently, no global assays are available to specifically identify BDUC.¹¹

A recent consensus report by the European Hematology Association (EHA) on the diagnosis of MBD has emphasized the limited clinical utility of global tests in MBDs, including thromboelastography, Platelet Function Analyzer (PFA)-100/200, and the skin bleeding time (BT), as well as thrombin generation (TG).¹⁰

A highly sensitive and specific single assay that detects any disruption in the hemostatic balance would be invaluable,^{12,13} especially if it could predict clinical outcomes in BDUC patients. The aim of this review is to provide an overview of currently available global hemostatic assays and their utility in the clinical setting in MBDs and specifically in BDUC.

Global Assays in MBDs and BDUC

Global hemostatic assays are applied for identifying individuals predisposed to bleeding or thrombotic events. In the realm of thrombosis, these assays have been investigated for pinpointing states of hypercoagulability and for predicting thrombotic risks.^{14,15} When it comes to bleeding disorders, global assays typically serve as preliminary screening methods prior to embarking on extensive diagnostic procedures.¹⁰ Conventional global tests like PT, TT, and APTT focus on the plasmatic aspects of coagulation. In addition, patients with mild deficiencies of coagulation factors VIII, IX, or XI may sometimes have normal APTT results. Therefore, specific factor analyses are essential in patients suspected of having CFD.^{16–18} Other assays consider factors beyond clotting proteins, including the influence of platelet function, blood flow dynamics on platelet–endothelium interactions, and the contribution of red blood cells to the stability of the clot formation, as illustrated in **Fig. 1**.^{10,19,20} However, these tests may not always detect disorders related to primary hemostasis or fibrinolysis, nor variations in natural anticoagulants.¹⁹

Over the past few decades, many global assays have been studied extensively in the context of MBD.^{21,22} Based on the *in vivo* skin BT test, which primarily evaluated primary hemostasis, a progression to *in vitro* global assays has been observed.²³ These include the PFA-100/200 tests, which

have been extensively researched in patients with PFD and VWD.²⁴ More recently, the focus has shifted to understanding TG in platelet-poor plasma and occasionally in whole blood, which can indicate alterations of the intrinsic coagulation capacity leading to abnormal fibrin clot production.^{25,26} Similarly, turbidimetric assays for plasma clot formation and lysis have been employed to identify anomalies in the structure of fibrin clots in patients with bleeding disorders.^{26,27} In addition, point-of-care assays such as thromboelastography have emerged as an essential tool for effectively monitoring and managing treatment in patients experiencing significant bleeding events, such as trauma or postpartum hemorrhage (PPH).^{28,29} Additionally, assays like euglobulin clot lysis time (ECTL) and fluorescent plasmin generation (PG) have been applied in analyzing the complex process of fibrinolysis within bleeding pathologies.³⁰ Flow-based assays, utilizing flow chambers and microfluidic technology, have provided new insights into the mechanisms of blood flow and vascular injury.^{31,32}

In cases of BDUC, these global assays assist in deciphering the underlying pathological mechanism, whether it is due to abnormal plasma clot formation from defective coagulation factors, impaired interactions between platelets and the endothelium under shear stress, or conditions of hyperfibrinolysis. Their role as a diagnostic tool in clinical practice is still unclear and will need further investigations.

In this review, we will comprehensively discuss the array of global hemostatic assays that have been evaluated in the context of MBD and BDUC. Our objective is to critically assess their diagnostic value in identifying bleeding disorders and detecting deficiencies in the crucial hemostatic processes of primary hemostasis, plasmatic coagulation, and fibrinolysis, as presented in **Fig. 2**.

Skin Bleeding Time

Skin BT according to Duke or Ivy (using a blood pressure cuff to maintain constant pressure) is a global test that involves making incisions, typically on the forearm, and measuring the time it takes for clotting to occur.^{23,33} Originally, it served as a screening tool for patients with VWD and PFD.³⁴ However, this method is rarely used today due to its invasiveness, time-consuming nature, and the need for trained personnel.

While BT is reliable in severe cases of PFD, like Glanzmann thrombasthenia or Bernard-Soulier syndrome, and severe VWD (e.g., type 3), its sensitivity and specificity decreases in patients with milder phenotypes, such as mild PFD or VWD type 1.^{33,34} As a result, BT is not recommended as a screening test for MBDs.¹⁰

Data on BT in BDUC patients are limited to one study by Quiroga et al who analyzed BT in BDUC patients with mucocutaneous bleeding and found no difference between patients with an established diagnosis such as PFD or VWD and those with BDUC.³⁵ Nevertheless, BT was the only pathological finding in 35.8% of patients with BDUC. Overall, the BT showed low sensitivity, regardless of the diagnosis (established MBD or BDUC),³⁵ and its application is not recommended in BDUC patients.¹¹

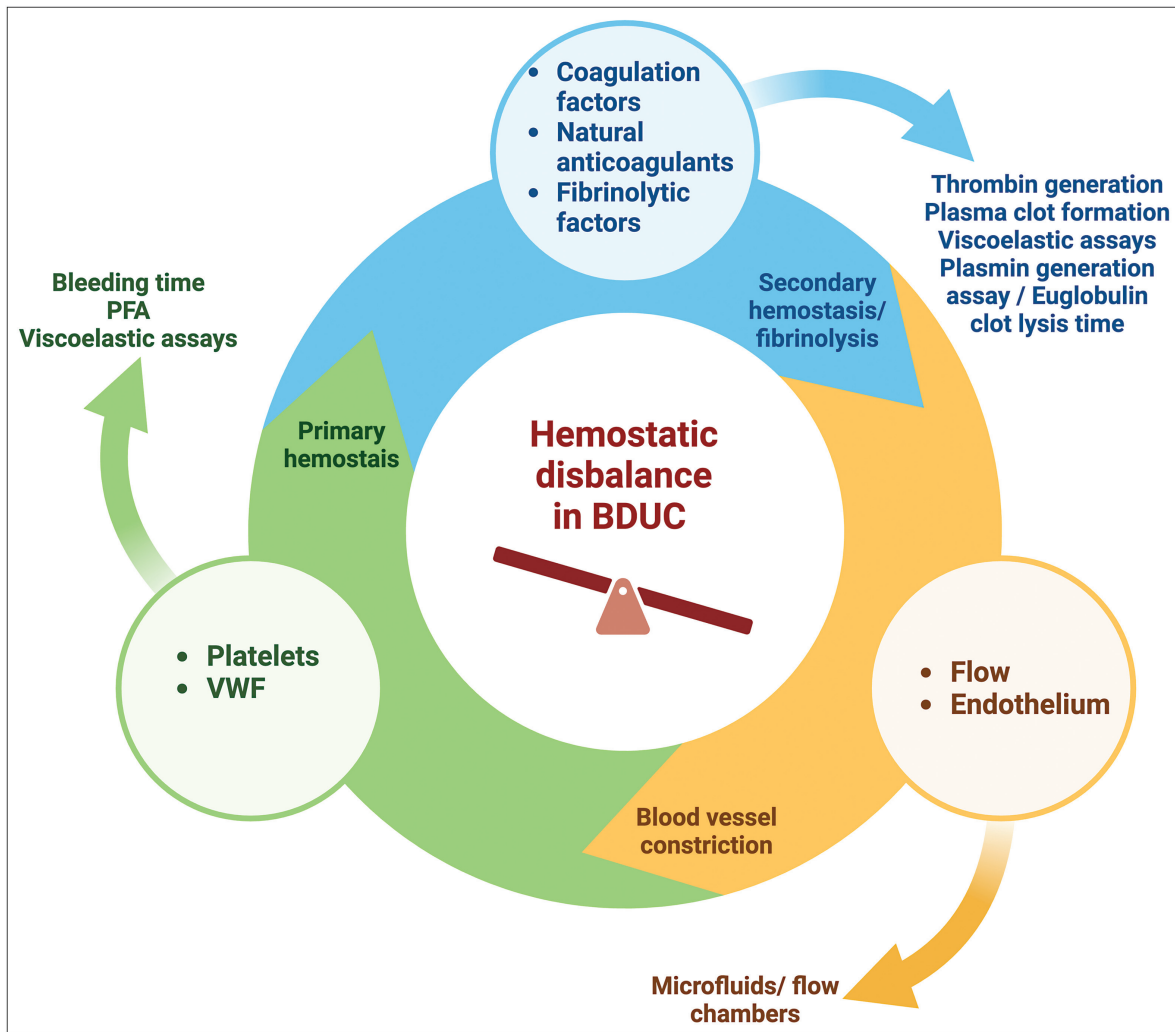


Fig. 1 Potential causes for imbalanced hemostatic capacity in BDUC patients. BDUC, bleeding disorder of unknown cause; PFA, platelet function analyzer; VWF, von Willebrand factor. (Created with BioRender.com.) [rerif].

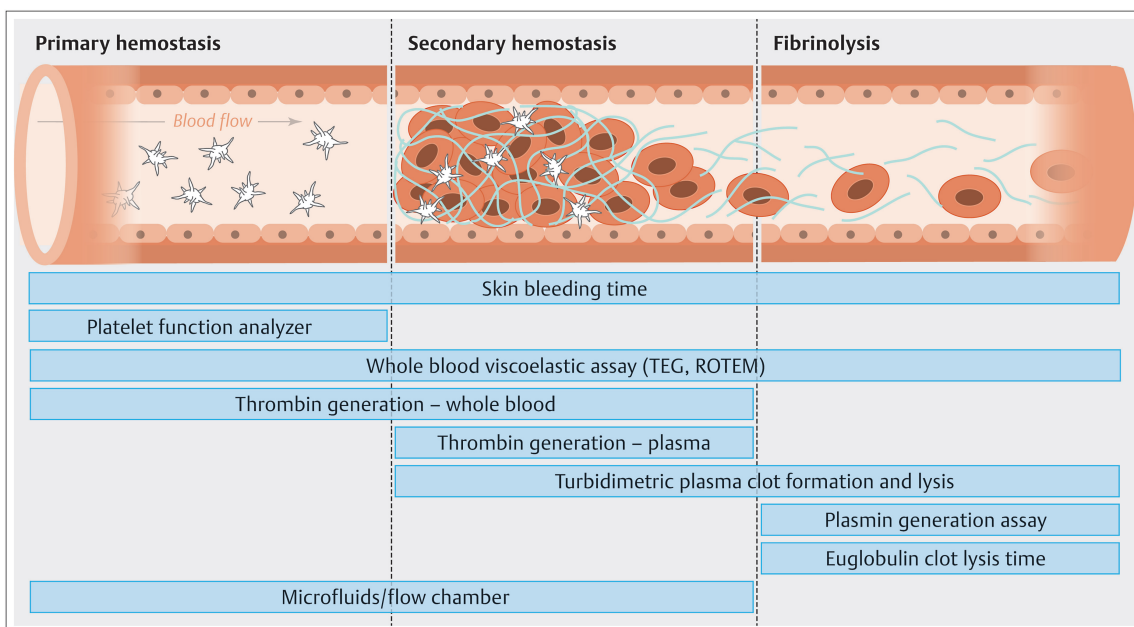


Fig. 2 Overview of global assays. ROTEM, rotational thromboelastometry; TEG, thromboelastography. (Created with BioRender.com) [rerif].

Platelet Function Analyzer (PFA-100/200)

PFA is a simple assay based on a microprocessor-controlled instrument utilizing disposable test cartridges.³⁶ Two commonly used cartridges are PFA collagen/epinephrine and PFA collagen/adenosine diphosphate. As the blood flows through the cartridge, platelets are activated by the agonists and form a platelet plug that occludes the aperture in the membrane. The technique involves measuring closure times (CTs), representing the duration until full occlusion of the aperture. Subsequently, the CT values are compared with established in-house reference ranges for each specific cartridge. In contrast to other assays, PFA also reflects the role of high shear forces required for unfolding VWF multimers to expose the VWF-A1 binding site for platelet GPIb.³⁷ Nevertheless, many underlying factors including hematocrit, platelet count, VWF levels, or ABO blood type may influence the CT,^{38,39} which limits its sensitivity. Overall, this whole blood assay reflects both primary and secondary hemostasis; therefore, the assays have been often declared as the *in vitro* skin BT. When using the PFA, it is crucial to rule out factors such as low hematocrit or the influence of medications like aspirin, which could extend CT.²⁴

PFA-100 has been explored as a preliminary screening test for primary hemostasis defects, as indicated by early data.^{40,41} In particular, PFA-100 has shown remarkable sensitivity in identifying VWD, when the VWF level is below 30 IU/dL,^{42–44} and in monitoring the efficacy of Desmopressin (DDAVP; 1-Desamino-8-D-Arginin-Vasopressin) treatment in VWD patients.^{42,45} However, it is important to note that the current guidelines do not support the use of PFA for VWD screening,⁴⁶ as the sensitivity and specificity for mild VWD are insufficient. PFA-100 demonstrates high accuracy in severe PFD, such as Glanzmann thrombasthenia and Bernard-Soulier syndrome.^{41,42,47} Nonetheless, its diagnostic value for mild PFDs remains a topic of debate, with conflicting results reported.^{40,41,48–51}

Quiroga et al showed a low ability of PFA-100 to detect BDUC patients with mucocutaneous bleeding and a positive family history. In patients from the VIBB, two-thirds of BDUC patients had prolongations in any PFA-100 CT with epinephrine or adenosine diphosphate, as recently presented by our group. Nevertheless, we could show that prolonged CTs were less common in BDUC than in patients with known defects of primary hemostasis.⁵²

The PFA remains the only shear-dependent assay used clinically, as also shown by Heubel-Moenen et al and discussed later in this review.³² Based on existing data, the PFA should not be relied upon for screening purposes of MBD because it may fail to detect mild PFD and VWD.

Overall, the PFA-100 assay is currently not recommended in the diagnostic workup of MBDs,¹⁰ but may be used in research studies.¹¹

Viscoelastic Assays

Viscoelastic tests, which are performed on a whole blood sample and offer a holistic view of *ex vivo* coagulation, provide results within minutes and can therefore be viewed and evaluated at the point-of-care.⁵³ The most established

of these tests are thromboelastography (TEG 5000) and rotational thromboelastometry (ROTEM delta).⁵³ In TEG, whole blood and clotting activators are mixed in a disposable cup heated to 37 °C and subjected to slow periodic and alternating rotations. A disposable plastic pin, suspended in the center of the cup, is enveloped by the developing clot. This adhesion to the pin and cup walls generates torque forces that displace the pin as the cup oscillates, and the forces are measured by a transducer. In the ROTEM platform, whole blood and coagulation activators are introduced into a stationary disposable cup that is also heated to 37 °C. Within the cup, a pin is suspended on a slowly oscillating spring-driven axis (4.75-degree arc, once every 6 seconds). This oscillating axis has an attached mirror onto which a beam of light is directed. As the clot forms around the pin, the oscillation of the pin decreases and changes in light reflection are detected by a photodetector.⁵⁴ More recently, portable point-of-care analyzers have been developed, such as Sonoclot, Quantra, and ClotPro, which provide specific clot quality parameters related to coagulation factors, fibrinogen/fibrin, platelets, and clot lysis.⁵⁵ Attempts have been made to also assess platelet function using viscoelastic testing and a specialized test called “thromboelastographic platelet mapping” has been developed, but its comparison with existing platelet function methods yielded conflicting results.⁵⁶

Viscoelastic testing is used in a variety of clinical settings and provides valuable insight into various medical scenarios, including presurgical assessment, trauma-related coagulopathy, PPH management, cirrhosis procedures, and critical care.^{29,57} Viscoelastic testing can also identify different states of fibrinolysis in blood, including hyperfibrinolysis, physiologic fibrinolysis, and fibrinolysis shutdown.⁵⁸

Viscoelastic assays have also been extensively studied as a diagnostic tool for MBDs,⁵⁶ including VWD^{59,60} and CFD such as hemophilia A/B,^{56,61} but the partly conflicting results did not lead to the implementation of these assays in the diagnostic workup of these bleeding disorders.

In BDUC, Veen et al reported no differences in ROTEM parameters between 121 patients with BDUC compared with 76 healthy controls. In comparison to 60 patients with other MBDs, BDUC patients had shortened clot formation time and increased maximum clot firmness.⁶² On the other hand, Wieland et al did not identify significant differences in ROTEM delta parameters of 100 patients with BDUC compared with 97 patients with other MBDs or 20 patients with a systemic disorder (e.g., Crohn's disease) and most patients' results were within the established reference ranges.⁶³ Similarly, MacDonald et al found ROTEM (NATEM) not significantly altered in their study of 124 BDUC patients, with only 9% showing prolonged clotting time or maximum lysis.⁶⁴

We recently presented data of ROTEM measurements from the VIBB at the ISTH 2022. We identified a higher maximum clot firmness and lower maximal lysis compared with PFD patients. On the other hand, in line with the results by Veen et al, BDUC patients had shorter clotting time and clot formation time, and higher maximum clot firmness compared with CFD patients. Comparison to healthy controls has not been performed to date.⁶⁵

Although viscoelastic assays offer a dynamic and comprehensive view of hemostasis by monitoring clot formation and breakdown in real time, making them suitable for point-of-care testing, our preliminary data require further confirmation. This is necessary before we can endorse their utility as a clinical assay for patients with BDUC.

Thrombin Generation

The TG assay is a method used to assess thrombin activation by measuring the cleavage of a fluorescent substrate, usually in platelet-poor plasma, which generates a TG curve.^{66,67} Coagulation is induced by adding tissue factor (TF) and phospholipids. Several parameters can be derived from a TG curve, including thrombin burst time, the maximum amount of thrombin generated (peak thrombin), TG rate, and the total amount of thrombin produced, also known as the endogenous thrombin potential (area under the curve [AUC]).⁶⁸ TG has been studied in different diseases such as (recurrent) venous thromboembolism,^{69,70} or disease severity of advanced chronic liver disease.⁷¹

TG demonstrates a better correlation with bleeding severity than factor levels in hemophilia A and B, and is also indicative of bleeding risk in FXI deficiency and VWD.^{72–76} In VWD, treatment with DDAVP has been shown to normalize TG, highlighting its potential as a monitoring tool for hemostatic therapies.⁷⁷

The TG assay not only assesses procoagulant factors but can also detect hemostatic abnormalities related to natural anticoagulants, such as alterations in thrombomodulin (observed in thrombomodulin-associated coagulopathy) or TF pathway inhibitor (seen in factor V–short disorders).^{19,20,78–81}

Thomas et al⁷⁸ have comprehensively discussed the utility of TG in patients with BDUC, as also summarized in **Table 1**. To date, a total of seven studies investigated TG in BDUC patients.^{25,26,62,82–85} Among them, four studies yielded negative findings, indicating no significant differences between BDUC patients and controls or abnormalities observed in less than 5% of BDUC patients. In contrast, three studies, including data from the VIBB, revealed substantial abnormalities when comparing BDUC patients to healthy controls or a

Table 1 Analysis of thrombin generation in distinct cohorts with BDUC patients

Study	No. of patients	Assay	Tissue factor concentration	Results
Ay et al ²⁵	101	Technothrombin (Technoclone)	5 pM	No significant difference between patients and healthy controls
Holm et al ⁸²	179	Methodology per Varadi et al ¹¹²	17.9 pM	Weak correlation between bleeding score and thrombin generation in females. Prolonged lag time in 6 patients (3%). Decreased peak thrombin generation in 4 patients (2%)
Hofer et al ²⁶	382	Technothrombin (Technoclone)	<0.3 pmol/L	Prolonged lag time, time to peak, and decreased maximum thrombin generation, velocity index, and area under the curve in BDUC patients compared with healthy controls
Alves et al ⁸³	45	Technothrombin (Technoclone)	Not specified	No significant difference between patients and healthy controls
MacDonald et al ⁸⁴	76 (high-dose TF) 52 (low-dose TF)	Thrombinoscope software (Diagnostica Stago)	High dose: 5 pM Low dose: 1.5 pM	High-dose TF: 17% patients had prolonged lag time, 12% had decreased endogenous thrombin potential. Low-dose TF: 26% had prolonged lag time, 19% had decreased endogenous thrombin potential
Veen et al ⁶²	121	Calibrated automated thrombogram assay (Diagnostica Stago)	1 pM	Prolonged lag time in BDUC patients, but no difference from healthy controls after adjustment for age, sex, BMI, platelet count fibrinogen, FXIII
Cornette et al ⁸⁵	59	ST Genesis and calibrated automated thrombogram (Diagnostica Stago)	TF: “low levels”	62.7 and 69.5% of BDUC showed abnormal thrombin generation using ST Genesis and calibrated automated thrombogram, respectively

Abbreviations: BDUC, bleeding disorder of unknown cause; BMI, body mass index; TF, tissue factor.
Source: Adapted from Thomas et al.⁷⁸

reference range. This diversity in study methodologies, as shown in ▶Table 1, with variations in TG assays and TF concentrations (which can affect sensitivity to coagulation defects) may underlie the inconclusive results. Also, BDUC cohorts display heterogeneity in terms of bleeding phenotype, severity, and inclusion criteria.⁵

The potential of TG to explore alterations in plasmatic coagulation, especially in BDUC patients, is notable. Nevertheless, the absence of standardization and conflicting evidence in existing literature necessitates caution, making it too soon to endorse these assays for standard clinical use.

Turbidimetric Fibrin Clot Formation Analysis

The analysis of clot formation and fibrinolysis provides valuable insights into hemostatic capacity. This can be assessed by turbidimetric assessment of plasma clot formation and lysis after the addition of calcium chloride (CaCl₂), TF, phospholipids, and tissue plasminogen activator (tPA).^{26,27}

Altered clot characteristics have been reported both in thrombotic^{86–89} and bleeding disorders such as hemophilia A and B.^{90–92} We previously have demonstrated that BDUC patients have a lower clot formation rate, resulting in a longer time to peak with an increased absorbance, and a shorter clot lysis time than in healthy controls. In contrast to our finding, Veen et al found in the earlier-mentioned study a significantly longer CLT compared with healthy controls.⁶²

In 52 women with BDUC and heavy menstrual bleeding, looser plasma fibrin clots reflected by a lower absorbance and shorter clot lysis time were found when compared with healthy controls.⁹³ It remains unclear if these results reflect a hyperfibrinolytic state, as Wiewel-Verschueren et al showed no signs of hyperfibrinolysis in heavy menstrual bleeding by measuring fibrinolytic parameters.⁹⁴

The lack of conclusive data on plasma clot formation assays in BDUC currently limits their clinical applicability. However, the assay can serve as a valuable research tool and its association with other plasmatic abnormalities can be investigated.¹¹

Plasmin Generation Assays

Clot formation is counter-balanced by the fibrinolytic system, but measuring fibrinolysis in vitro poses challenges.⁹⁵ Plasmin is the key fibrinolytic parameter responsible for cleaving fibrin and generating fibrin degradation products, such as D-dimer. Several research groups have developed assays to assess PG capacity in plasma.³⁰ PG assays rely on the consumption of a fluorescent substrate and both coagulation and fibrinolysis are triggered through the addition of TF, tPA, phospholipids, and CaCl₂, as comprehensively reviewed by Miszta et al.³⁰ Notably, recently Wolberg et al have developed a PG assay to correct for the inner filter effect and substrate consumption, which is particularly suitable for use in both mouse and human plasma.⁹⁶

Most studies have investigated PG in the context of severe bleeding disorders such as severe hemophilia,⁹⁷ rare CFDs,⁹⁸ or PAI-1 deficiency.⁹⁹ There is a notable lack of studies of PG in MBD and BDUC patients.

In our recent findings presented at the ISTH conference 2023, we observed a paradoxical reduction in PG potential, with lower peak plasmin in 375 BDUC patients compared with 100 healthy controls (ISTH 2023, unpublished data).¹⁰⁰ This counterintuitive decrease in PG might be attributed to counterregulatory mechanisms, as also seen by increased α 2-antiplasmin and TAFI in BDUC patients.^{94,101,102} Also altered clot structure with suspected thicker fibers in BDUC patients might influence PG, supported by findings from Longstaff et al, who documented altered tPA activity dependent on fibrin fiber structure.¹⁰³

Euglobulin Clot Lysis Time

The ECLT test, which reflects overall fibrinolytic activity in plasma, involves diluting plasma with acetic acid and incubating it on ice for 15 minutes.¹⁰⁴ During this incubation, a precipitate forms, known as the euglobulin fraction of plasma, which contains plasminogen, plasminogen activators (primarily t-PA), and fibrinogen. After incubation, the mixture is centrifuged again at 4 °C and the supernatant is discarded, and the precipitate is dissolved in a buffer solution. This mixture is then induced to clot using thrombin, and the time for clot lysis is determined through visual inspection at 15-minute intervals. It is crucial to perform a parallel test with a control plasma sample collected simultaneously.^{105,106}

Overall the sensitivity of the ECLT for a deficiency of fibrinolytic inhibitors is limited, as the euglobulin fraction reduces the levels of some fibrinolytic factors, including α 2-antiplasmin (7.1% recovered in the euglobulin fraction), TAFI (38.5%), PAI-1 (42.2%), and tPA (90.8%), which tips the balance toward remaining profibrinolytic factors.^{95,106} While in a high proportion of trauma patients the ECLT has shown a fibrinolytic activation,¹⁰⁷ data on the utility of ECLT in hereditary bleeding disorders have shown to be limited.

Valke et al conducted a study with 160 BDUC patients having a high Toretto bleeding assessment score >10.¹⁰⁸ In the study by Valke et al, they measured ECLT before and after venous compression, computing the ratio between these two measurements. Their study revealed aberrant fibrinolytic profiles in ~39% of the cohort, indicated by elevated ECLT ratios or diminished baseline ECLT in about half of these cases. The remainder presented with reduced levels of PAI-1 antigen and activity.¹⁰⁸

Interestingly, there was no difference in bleeding severity between patients with abnormal fibrinolysis findings and those without such abnormalities.

Flow Chamber/Microfluids

Flow-based in vitro assays are extensively employed to explore the involvement of platelets and coagulation as well as determine the impact of specific factors or surface receptors in hemostasis and thrombosis.¹⁰⁹ Their primary advantage compared with other assays lies in their ability to incorporate blood flow, which governs numerous facets of platelet function, encompassing adhesion, activation, and aggregation. Blood flow also plays a central role in regulating coagulation by influencing the local concentrations of coagulation factors

within and around thrombi.¹¹⁰ Microfluidic systems are utilized to explore thrombus formation by flowing whole blood over immobilized fibrillar collagen, closely mimicking the in vivo process that occurs after vascular injury.¹⁰⁹ In this setup, platelets adhere, become activated, and initiate the formation of a mural thrombus. This process can also be examined under conditions of TG, particularly by reintroducing calcium to blood collected in sodium citrate. Blood can be perfused over different surfaces at different flow rates, which allows discrimination of hemostatic functions in response to different surfaces/agonists as well as at high versus low shear.³¹ Commonly used microfluidic devices provide a laminar blood flow pattern through rectangular or square channels, but flow chambers can also be designed to mimic vessel branching and turbulent shear stress. In this way, they allow for a broad range of applications and the experimental setup can be adjusted to the research question. Real-time imaging of these video-microscopic in vitro thrombus formation assays has been employed in a wide range of basic studies, which include screening for hereditary or acquired platelet-related pathologies as well as assessing the effectiveness of novel antiplatelet treatments.¹¹¹ In this way these systems could help narrow down defects in primary as well as secondary hemostasis in patients with BDUC.

Brouns et al showed that flow perfusion measurements under coagulating conditions, such as with collagen/TF surfaces, can also differentiate between mild, moderate, and severe hemophilia, aligning with the patient's bleeding risk.¹¹¹ In a study conducted by Heubel-Moenen et al, 14 patients with BDUC and prolonged CT in PFA-100 were examined using microfluidic tests and compared with a control group of healthy individuals. The investigation unveiled a reduction in platelet adhesion and thrombus formation in BDUC patients. Additionally, these abnormalities were associated with lower integrin activation, impaired phosphatidylserine exposure, and reduced P-selectin expression. Notably, the study highlighted that five patients with the most severe microfluidic irregularities also exhibited results of the lowest normal range of light transmission aggregation analysis. Collectively, the findings suggest that microfluidic analysis can identify shear-dependent PFDs, which might remain undetected by conventional platelet function assays in BDUC patients.³²

Conclusion

As BDUC is a diagnosis of exclusion,¹¹ exhaustive investigations of plasmatic coagulation and platelet function are required, a process often limited to tertiary centers due to its diagnostic challenges and the need for specialized expertise. While the use of global hemostatic assays has been extensively studied in some MBDs like VWD or PFD, limited data exist on their application of most assays in the context of BDUC. Nevertheless, global assays hold the potential to serve as confirmatory tests for BDUC patients, especially those not assessed in specialized centers, and therefore offer a valuable diagnostic tool. Moreover, they shed light on crucial aspects of hemostasis often overlooked in traditional laboratory analyses such as shear stress-dependent platelet-endothelial interaction and hyperfibrinolysis. Although

not currently recommended for clinical decision-making, these assays could serve as promising research tools for the sub-categorization according to the pathophysiological mechanism (e.g., hyperfibrinolysis or defect of primary hemostasis) of BDUC patients and guiding further scientific investigations.

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Conflicts of Interest

D.M. received honoraria for advisory board meetings and lectures from CSL Behring., A.A. declares no conflict of interest. J.G. received honoraria for lectures and advisory board meetings, and research funding for the Medical University of Vienna from CSL Behring, Novartis, Amgen, Sobi, and Takeda.

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