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Fibrinolysis and bleeding of unknown cause

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Abstract

Patients with bleeding of unknown cause (BUC) present with a variety of mild to moderate bleeding symptoms, but no hemostatic abnormalities can be found. Hyperfibrinolysis is rarely evaluated as the underlying cause for bleeding in clinical practice, and well-established global assays for abnormal fibrinolysis are lacking. Few patients with definitive fibrinolytic disorders, including α2-antiplasmin deficiency, plasminogen activator inhibitor 1 deficiency, or Quebec platelet disorder, have been reported. This review aims to summarize data on established fibrinolytic disorders and to discuss assessments of fibrinolysis in prior bleeding cohorts. Furthermore, we review available global tests with the potential to measure fibrinolysis, such as turbidity fibrin clot assays and rotational thromboelastometry, and their relevance in the workup of patients with BUC. We conclude that, due to the lack of adequate global tests, hyperfibrinolysis might be an underdiagnosed cause for a bleeding disorder. The diagnosis of hyperfibrinolytic bleeding disorders would improve patient care as effective treatment with antifibrinolytic agents is available.

KEYWORDS
bleeding, blood coagulations disorders, fibrinolysis, plasminogen activators, plasminogen inactivators

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Essentials

- The vast majority of patients with mild bleeding tendencies are patients with bleeding of unknown cause (BUC).
- Hyperfibrinolysis is a possible underlying cause for bleeding in patients with BUC.
- Due to lack of sensitive assays, abnormalities in fibrinolytic factors may be underdiagnosed.
- Diagnosing hyperfibrinolysis enables targeted treatment with antifibrinolytics.

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1 | INTRODUCTION

Bleeding manifestations in mild to moderate bleeding disorders (MBDs) are generally mild but can also be life-threatening under certain circumstances, for example, in cases of hemostatic challenges such as surgery or childbirth.[1] Major spontaneous bleeding rarely occurs, and mild bleeding symptoms such as easy bruising and mucocutaneous bleeding are predominant. In a large proportion of patients with MBDs, no diagnosis can be found despite thorough laboratory investigations.[2-4] These patients are classified as having bleeding of unknown cause (BUC) and can present with a wide range of symptoms from easy bruising, epistaxis or menorrhagia to severe bleeding complications after surgery or giving birth.[5,6] Patients with BUC are indistinguishable from patients with a mild to

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moderate bleeding phenotype and an established diagnosis, such as platelet function defects, coagulation factor deficiencies, or low von Willebrand factor (VWF) levels, in respect to their bleeding manifestations and bleeding score.[3,4,7] However, the inability to identify the etiology of the bleeding disorder prevents use of targeted treatments and places a high psychological strain on patients and treating physicians.

Hyperfibrinolysis has been considered as a possible mechanism underlying BUC, as hyperfibrinolysis is not detected in global coagulation tests, and parameters of fibrinolysis are generally not included in the basic laboratory workup of patients with MBD. According to recent recommendations, the second step of investigations should evaluate for disorders of the fibrinolytic system[1]; however, exact guidance for the laboratory assessment of hyperfibrinolysis is lacking.[5]

In this review, we summarize current knowledge on established hyperfibrinolytic bleeding disorders and hyperfibrinolysis as a possible cause for the bleeding tendency in patients with BUC. Furthermore, we provide insights into the current approaches and challenges of assessing fibrinolysis in patients with MBD or BUC.

2 | MEASURING FIBRINOLYSIS

The importance of the fibrinolytic system for the hemostatic balance was recognized early.[8] However, due to the lack of well-established methods to globally assess fibrinolysis or involved fibrinolytic factors and the paucity of clinical data, its clinical relevance with respect to bleeding disorders has not been fully elucidated.[9]

Generally, the diagnosis of hyperfibrinolysis is based on either a deficiency of fibrinolytic inhibitors or an increase in activator enzymes that further increase plasmin generation.[9]

Plasmin is the central protein of fibrinolysis and its activation is tightly regulated by a complex system of activators and inhibitors (Figure 1).[9,10] Plasminogen, the proenzyme of plasmin, is attached to C-terminal lysine residues of fibrin and fibrin degradation products during clot formation. Plasminogen can be converted by both tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) to plasmin. Both activators are primarily secreted by endothelial cells, renal epithelium, and monocytes.[10] There are several other cellular sources of tPA and uPA that might be considered relevant. For example, hepatocytes contribute to basal circulating tPA activity,[11] and an increased secretion of uPA was found in human lung microvascular endothelial cells.[12] Also, tPA of keratinocytes was found to contribute to cell-surface–associated plasminogen activation in cell line experiments.[13] Additionally, mouse experiments showed that neurons such as oligodendrocytes, ependymocytes, and mastocytes produce tPA to some extent.[14]

The binding of tPA and plasminogen to C-terminal lysine residues on fibrin leads to plasmin activation and fibrin degradation. This process induces generation of new C-terminal lysine residues, which initiates a positive feedback loop. By increasing the availability of binding sites, a conformational change of plasminogen is initiated,

which then leads to facilitated activation by tPA. Alongside that, uPA is also able to activate plasminogen, but has a lower affinity to fibrin.[10,15] Plasminogen activator inhibitor-1 (PAI-1) is an important inhibitor of both activators, tPA and uPA.[10] In addition, plasmin itself is inhibited by both α2-antiplasmin and, to a lesser extent, α2-macroglobulin.[10] Another fibrinolysis inhibitor, thrombin-activatable fibrinolysis inhibitor (TAFI),[10] is activated by the thrombomodulin-thrombin complex and removes the carboxy-terminal lysine residues from fibrin. This limits plasmin generation by interrupting the positive feedback loop and leads to more stable fibrin thrombi in vitro.[10,15]

As the key enzyme in fibrinolysis, plasmin's primary function is the degradation of fibrin polymers,[16] which generates fibrin degradation products (eg, D-dimer).[10] Structural properties of fibrin clots determine the rate of fibrinolysis; generally, clots with thinner fibers are denser and are lysed more slowly.[17] Nevertheless, with defects in the fibrinolytic system, thinner fibers can be lysed faster than thicker fibers.[17]

The diagnosis of hyperfibrinolysis is challenging due to the paucity of available and routinely used specific laboratory tests.[18] In contrast to coagulation, which can be measured by standardized coagulation assays (eg, activated partial thromboplastin time [aPTT] and prothrombin time [PT]), assessment of dysregulated fibrinolysis is difficult in vitro.[9,19]

Several global screening assays have been proposed that could potentially screen for hyperfibrinolytic activity; the most common ones are summarized in Table 1.

One major challenge with assessing fibrinolysis is that fibrinolysis in vivo is very slow, and fibrinolytic activators need to be added or inhibitors of fibrinolysis need to be reduced to induce fibrinolysis in vitro (Table 1). For example, for turbidimetric measurements in plasma-based systems, tPA is added to induce fibrinolysis in vitro, which subsequently reduces the sensitivity to levels of endogenous plasminogen activators.[9] In contrast, the preparation of the euglobulin fraction reduces the levels of some fibrinolytic factors, including α2-antiplasmin (7.1% recovered in the euglobulin fraction), TAFI (38.5%), PAI-1 (42.2%), and tPA (90.8%),[9,20] which tips the balance toward remaining profibrinolytic factors. Consequently, the sensitivity of the euglobulin clot lysis time (ECLT) for a deficiency of fibrinolytic inhibitors is limited.[9]

Based on a recent study by Ilich et al,[19] the traditional ECLT with adapted spectrophotometric readouts appears to better assess hyperfibrinolysis. This was achieved by reducing inhibitor levels in the euglobulin fraction below a threshold such that the assay still detects fibrinolytic activity. The threshold for active PAI-1 to keep its inhibitory function and, consequently, shorten the ECLT was 20-25 nM. Furthermore, as low fibrinogen was previously a major limitation in ECLT assays, the addition of plasminogen-free fibrinogen improved the sensitivity to alterations of fibrinolytic factors, such as low PAI-1 levels.[19] Data have further shown a strong correlation between ECLT and tPA or α2-antiplasmin levels, but not TAFI levels.[19]

Another clinically applied diagnostic test is rotational thromboelastometry (ROTEM), which is often used as a point-of care test

FIGURE 1 Schematic overview of activators and inhibitors of fibrinolysis. PAI, plasminogen activator inhibitor; sTM, soluble thrombomodulin; TAFI, thrombin-activatable fibrinolysis inhibitor; tPA, tissue-type plasminogen activator; TXA, tranexamic acid; uPA, urokinase-type plasminogen activator
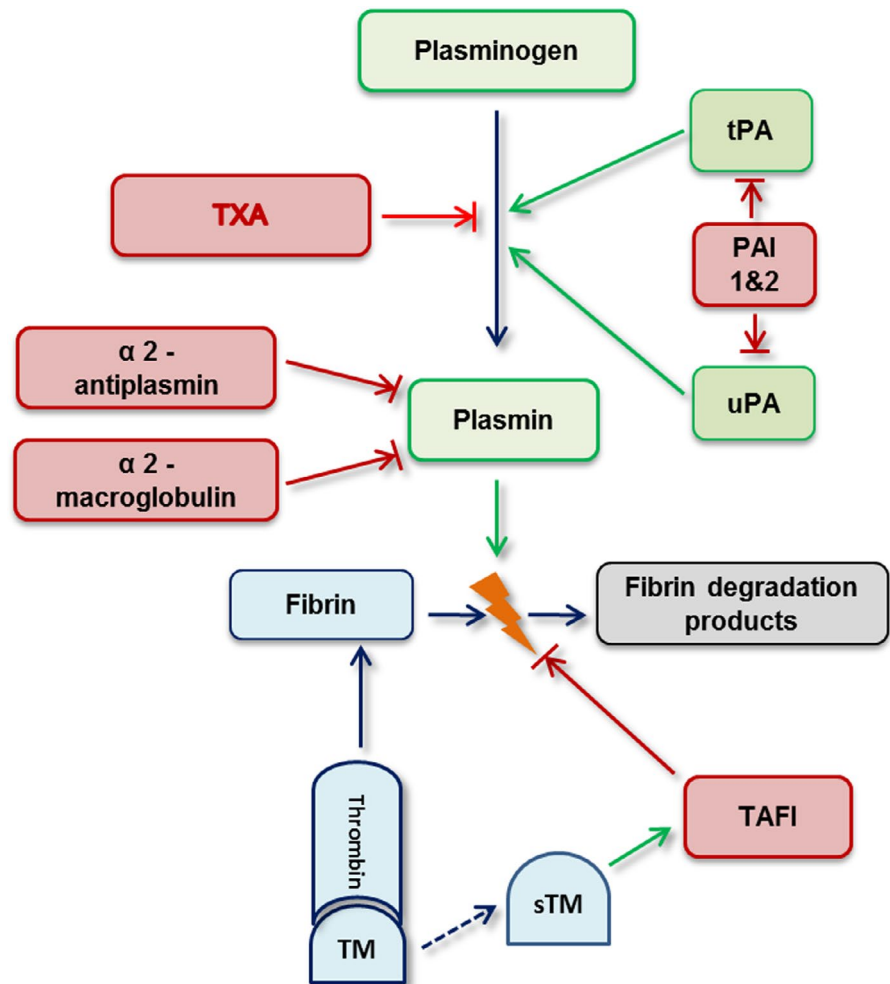

TABLE 1 Global tests for measuring fibrinolysis

Method	Sample type	Induction of coagulation	Induction of lysis	Measurement	Increased fibrinolysis	Sensitivity
ECLT[20,62]	Plasma	Thrombin	Endogenous tPA contained in the euglobulin fraction	Optical density: time to visual lysis	ECLT <60-120 min	Low sensitivity for TAFI and α2-antiplasmin deficiencies
TEG and ROTEM: EXTEM and APTEM[9,63]	Whole blood	Tissue factor Calcium Phospholipids (EXTEM and APTEM) Aprotinin (APTEM, inhibition of fibrinolysis)	-	Changes in viscosity	Lysis index (proportional amplitude height of maximum clot firmness after 60 or 30 min) <85%	All fibrinolytic factors
Turbidimetric clot formation and lysis assay[45,64]	Plasma	Tissue factor Phospholipids Calcium	tPA	Optical density (turbidimetric analysis of dynamic changes)	No established cutoff of clot lysis time	Not sensitive for tPA alterations

ECLT, euglobulin clot lysis time; ROTEM, rotational thromboelastometry; TAFI, thrombin-activatable fibrinolysis inhibitor; TEG, thromboelastography; tPA, tissue-type plasminogen activator.

(eg, during surgery) and does not require the addition of a fibrinolytic activator (eg, tPA). However, this test has not been validated as a diagnostic tool in patients with MBDs because results within the normal range do not reliably rule out a hyperfibrinolytic disorder.[1,9,18]

Altogether, there is no gold standard assay for the assessment of the fibrinolytic system, as each test has limitations and thus these assays are primarily used as research tools.[21]

3 | ESTABLISHED HYPERFIBRINOLYTIC BLEEDING DISORDERS

Alterations in factors and regulators of the fibrinolytic system have been identified in both case series and families with a pattern of delayed bleeding after trauma or surgery and mucocutaneous bleeding, but detailed knowledge of fibrinolytic bleeding disorders remains incomplete. Saes et al[18] recently reviewed a patient series with bleeding disorders due to hyperfibrinolysis, including 14 homozygous patients and 104 heterozygous patients with α2-antiplasmin deficiency, 36 patients with PAI-1 deficiency, 23 patients with Quebec platelet disorder, and 4 patients with tPA excess, of whom in two cases the PAI-1 antigen levels and PAI-1 activity were within the normal range. It remains unclear to what extend PAI-1 levels in the 36 patients categorized as "PAI-1 deficient" are influenced by other factors, as only few genetic confirmations for PAI-1 deficiency have been described.[22-24]

Recently, Westbury et al[25] identified, in a family with a thrombomodulin-associated bleeding disorder, a stop-gain mutation in the *CPB2* gene that encodes TAFI and resulted in 50% lower TAFI levels. Consistent with mouse experiments that did not find an effect of TAFI deficiency on the hemostatic capacity,[26] subjects with only this genetic variation, but no thrombomodulin alterations, were asymptomatic.[25] To the best of our knowledge, no bleeding disorders related to a gain-of-function mutation in plasminogen have been described in humans.

The diagnosis of alterations of fibrinolytic factors is often very challenging, as screening tests do not reliably detect all disorders. Using a threshold-dependent method of the euglobulin fraction, plasma from patients with congenital PAI-1 deficiency resulted in a significantly shortened ECLT,[19] although ECLT has low sensitivity for α2-antiplasmin deficiency. Additionally, there are no established global assays that reliably detect tPA excess or Quebec platelet disorder. In Quebec platelet disorder, a disorder characterized by overexpression of uPA in alpha granules of platelets and excess plasmin generation, global coagulation screening tests such as the aPTT and PT, plasmatic levels of both coagulation and fibrinolytic factors (including uPA), and clot lysis measured by thromboelastometry are usually within the normal range.[27] In addition, bone marrow examination, platelet morphology, and the number of alpha granules and dense granules are normal.[27] Factor V (FV) and platelet FV can be slightly reduced, and aggregation with epinephrine and alpha-granule release are usually impaired,[18,27-29] which are unspecific findings. However, platelet perfusion studies have shown that addition

of platelets from patients with Quebec platelet disorder into a forming clot led to disruption of fibrin and platelet aggregates.[29] Overall, a definitive diagnosis of Quebec platelet disorder requires the determination of increased uPA in the platelet alpha granules and the genetic testing demonstrating a duplication mutation in the urokinase plasminogen activator gene (*PLAU*).[27,30]

Generally, the bleeding phenotype in patients with hyperfibrinolysis manifests as delayed posttraumatic bleeding, postsurgical bleeding, bleeding after dental procedures, menorrhagia, and epistaxis.[18] Interestingly, hyperfibrinolytic bleeding disorders are not only associated with bleeding manifestations but also with obstetric complications such as miscarriages, as reported in α2-antiplasmin and PAI-1 deficiency.[18]

4 | HYPERFIBRINOLYSIS IN BLEEDING COHORTS AND PATIENTS WITH BUC

Systematic investigations of hyperfibrinolysis as a cause for more severe bleeding phenotypes in patients with an established bleeding disorder or as the underlying cause for bleeding in patients with BUC have rarely been performed. Available data on fibrinolysis in bleeding cohorts are described in the following paragraphs and are summarized in Table 2. Due to major differences in key aspects of these studies such as bleeding phenotypes, study design, and demographic and sample size, comparability of the data on hyperfibrinolysis in bleeding cohorts is limited.

PAI-1 and tPA–PAI-1 complex levels were measured in 88 adult patients before and after cardiac surgery and lower preoperative PAI-1 and postoperative tPA–PAI-1 complex levels were associated with increased postsurgical bleeding.[31] One limitation of this study is that PAI-1 antigens were measured in a cardiac surgery population, which does not reflect patients with hereditary MBDs. Consequently, PAI-1 levels in all patients were within the normal range of 1-25 ng/mL. Generally, high PAI-1 levels prolong clot lysis time and are associated with an increased risk of venous and arterial thrombosis, and thus might prevent postsurgical bleeding.[32]

In a study evaluating low PAI-1 levels as a cause for bleeding, Agren et al[33] found lower levels of PAI-1 activity in 586 patients, mostly with a mild bleeding tendency, in comparison to 100 blood donors, and to 100 age- and sex- matched healthy controls. The prevalence of low PAI-1 levels, which was defined as PAI-1 activity ≤1 U/mL in this study, was 23% in patients, higher than in both control groups with rates of 13% and 10% in blood donors and healthy matched controls, respectively. In this study, there was no difference in tPA–PAI-1 complex levels between the groups. Low PAI-1 levels were not associated with more bleeding manifestations and/or bleeding severity. The study population consisted of a large but heterogenous group of patients, of whom 75% had a clinically relevant bleeding tendency. Patients were included regardless of their diagnoses, which included platelet function defects in 24.4%, von Willebrand disease (VWD) in 10.1%, thrombocytopenia in 1.2%, coagulation factor deficiencies in 2.7%, and BUC in 57% of patients.

TABLE 2 Results of the studies investigating hyperfibrinolysis in patients with bleeding disorders

	Bleeding symptoms	tPA antigen	PAI-1	tPA-PAI-1 complexes	α2-antiplasmin	TAFI	D-Dimer
Gruenewald et al[41]	Bleeding phenotype in severe congenital hemophilia	⇑	⇔	⇔	⇔	⇑	⇔
Agren et al[33]	Mild bleeding	nd	⇓ (blood donors) ⇓ (healthy controls)	⇔	nd	nd	nd
Ozolina et al[31]	Bleeding after cardiac surgery	nd	⇓	⇓	nd	nd	⇑
Szczepaniak et al[35]	Heavy menstrual bleeding	⇓	⇓	nd	nd	nd	⇔
Wiewel-Verschueren et al[36]	Heavy menstrual bleeding	⇔	⇔	nd	⇑	⇑	nd
Matus et al[42]	Hereditary mucocutaneous bleeding	nd	nd	nd	nd	⇑	nd
Szczepaniak et al[35]	Heavy menstrual bleeding	⇓	⇓	nd	nd	nd	⇔
Gebhart et al[34]	Bleeding of unknown cause	⇔	⇔	⇓	⇑	⇑	⇔
Vries et al[48]	Patients ≥1 bleeding symptom	nd	⇓	nd	⇑	⇔	nd

Note: ⇔no statistical difference; ⇑statistically significant increase; ⇓statistically significant decrease; nd, not done.
PAI-1, plasminogen activator inhibitor 1; TAFI, thrombin activatable fibrinolysis inhibitor; tPA, tissue-type plasminogen activator.

Unfortunately, data on the incidence of low PAI-1 activity in patients with specific MBDs and/or BUC were not reported.

In our study by Gebhart et al[34] of 270 patients with BUC within the Vienna Bleeding Biobank (VIBB), we did not identify alterations in the levels of tPA antigen or PAI-1 activity in comparison to 98 healthy controls. Low PAI-1 levels occurred in 34% of patients and 34% of controls. However, our study cohort differed in several aspects from the patients in the study by Agren et al[33] First, Agren et al did not include patients with epistaxis, gingival bleeding, and subconjunctival bleeding, as these were not classified as clinically significant bleeding by the authors. In our study, inclusion of patients was determined by experienced hemostasis experts. Second, our study excluded patients who had surgery or gave birth within the past 6 weeks, bacterial infection within the past 2 weeks, active malignancy, pregnancy, or thrombocytopenia, while these factors were not considered in the study by Agren et al. Furthermore, different assays were used for the measurement of PAI-1 and tPA–PAI-1 complex levels, which could affect comparability between these cohorts.

In a small study of 52 women with heavy menstrual bleeding, which excluded patients with uterine pathology, a definite bleeding disorder (including VWD or coagulation factor deficiencies), who used nonsteroidal anti-inflammatory agents, were thrombocytopenic ($<100 \times 10^9$/L), or had acute infections or severe comorbidities such as malignancies or renal insufficiencies, Szczepaniak et al[35] reported decreased levels of tPA antigen (5.96 vs 9.72 ng/mL) and PAI-1 antigen (8.54 vs 11.89 ng/mL) compared to 52 healthy controls. However, these findings could not be confirmed in a larger study of 102 women with heavy menstrual bleeding without a known bleeding disorder and a pictorial blood assessment chart score of >100. Wiewel-Verschueren et al[36] identified lower levels of PAI-1 antigen in their patients, only when comparing women with menorrhagia without gynecological abnormalities with women with gynecological abnormalities. In contrast, the above-mentioned study by Szczepaniak et al excluded women with gynecological abnormalities. Notably, the studies used ELISAs from different companies (American Diagnostica, Stamford, CT, USA; and Asserachrom Diagnostica Stago, Asnieres-sur-Seine, France), which have different reference ranges and thus are not directly comparable. The authors around Wiewel-Verschueren also intensively discussed the time of testing fibrinolytic parameters within the menstrual cycle. While Szczepaniak et al performed blood testing within 10 days after menstruation, Wiewel-Verschueren et al measured the fibrinolytic factors 1 week after menstruation. This is the period during the menstrual cycle when reserves of procoagulant factors have been used to compensate for the blood loss, which, according to the authors, could lead to a compensatory increase in production of fibrinolytic factors and thus might be the time with the highest levels of fibrinolytic factors during the menstrual cycle.[36]

In general, PAI-1 levels are rarely examined in bleeding patients, and commercially available ELISAs are primarily used for the evaluation of thrombotic disorders, where a correlation with high PAI-1 levels has been described.[32] It remains unclear whether the lower limit for PAI-1 levels is well defined in these ELISAs and can be used for determining pathologic PAI-1 levels.[33] Also, circadian variations,[37] age,[33] and body mass index (BMI)[38,39] as well as genetic polymorphisms[40] may influence PAI-1 activity[33] in patients with BUC. The diurnal variations of higher PAI-1 levels and lower tPA activity in the morning were addressed in most studies by blood drawing at the same time of day.[33,34] Also, the influence of sex, age, BMI, and estrogen was adjusted for in the statistical analysis by most studies.[33,34]

Whereas most of the studies investigated tPA antigen levels, albeit with conflicting results[35,36,41] (Table 1), tPA activity was investigated by our group only in a study cohort of patients with BUC. Nevertheless, increased tPA activity and decreased inhibition of tPA

by PAI-1 in patients with BUC, represented by lower tPA–PAI-1 complex levels,[34] may point toward increased fibrinolysis as a possible cause for the bleeding tendency in patients with BUC. Increased tPA activity in conjunction with lower levels of tPA–PAI-1 complexes indicate an impaired counterregulatory mechanism, which could lead to higher fibrinolytic activity.[34]

A study by Gruenewald et al investigated fibrinolytic parameters in patients with severe hemophilia and demonstrated that the bleeding phenotype correlated with parameters of the fibrinolytic system, specifically finding increased levels of tPA antigen and TAFI in patients with more severe bleeding phenotypes.[41] In this study, TAFI activity was determined using a commercially available ELISA that measures latent (activatable), activated, and already inactivated TAFI. The authors hypothesize that the lack of active thrombin in people with severe hemophilia, which is required for pro-TAFI conversion into its active form, prevents effective inhibition of fibrinolysis.

A paradoxical increase in TAFI was also identified in a study of 193 patients with MBDs (including 81.1% patients with BUC) who were between the ages of 4 and 48 years old and had mucocutaneous hemorrhage.[42] The study compared the bleeding patients with a control group of 143 subjects and found that increased TAFI levels were independent of the diagnosis (BUC, type 1 VWD, or platelet function defect).[42] This difference was independent of a diagnosis and persisted in a subgroup analysis of 116 patients with BUC. Similarly, in our study of patients with BUC,[34] we observed a paradoxical increase in the fibrinolysis inhibitors TAFI and α2-antiplasmin compared to healthy controls.[34]

Wiewel-Verschueren et al[36] also identified higher levels of TAFI and α2-antiplasmin in their cohort of women with heavy menstrual bleeding compared to healthy controls. Increases in TAFI in bleeding patients could be a counterregulatory mechanism to an existing bleeding tendency, for example, by balancing the augmented activity of tPA; however, the underlying mechanism remains unclear.[34,42]

In regard to global tests of fibrinolysis in patients with BUC, data are scarce, as most studies focused on well-described bleeding disorders such as hemophilia or VWD.[41,43-45] Szczepaniak et al[35] showed increased clot permeability and susceptibility to lysis in patients with heavy menstrual bleeding using a plasma clot turbidity assay with addition of tPA. This finding and its association with heavy menstrual bleeding demonstrates a possible contribution of altered clot parameters to the bleeding outcome. In our study, we found slower plasma clot formation in patients with BUC than in healthy controls, especially upon addition of exogenous tPA, but no difference in susceptibility to clot lysis.[46] In another study by our group of 382 patients with BUC, we found a lower clot formation rate and a shorter clot lysis time in patients with BUC compared to controls, indicating increased susceptibility of clots to fibrinolysis.[47] In contrast, Veen et al[4] recently reported a prolonged clot lysis time in a smaller cohort of 109 patients with BUC compared to healthy controls, and no difference in ROTEM parameters was found. Finally, another Dutch group, Vries et al,[48] recently did not identify significant differences in clot formation nor clot strength using ROTEM

nor differences in a turbidity assay between 240 patients with self-reported bleeding and 95 patients without bleeding symptoms who were scheduled for an elective surgery. The discrepancies between these studies may be partially explained by the smaller samples sizes in some cohorts,[4,47,48] the heterogeneity of investigated patients, and interlaboratory variations.[49] More specifically, Hofer et al[47] included adult patients with a MBD, who were screened by a hemostasis expert, Veen et al included subjects >12 years old while Vries et al compared patients >18 years old who had one or more self-reported bleeding symptom before an elective surgery with patients without a self-reported bleeding manifestation. Both Dutch studies analyzed ROTEM data that were acquired under the same conditions within 2 hours after blood collection. All three studies performed the plasma clot formation assays under the addition of recombinant tPA to induce fibrinolysis and according to recent recommendations.[49] However, it was recently found that for plasma clot turbidity assays, there is high interlaboratory variability (with coefficients of variability up to 50%), even when the same protocols are used.[49]

Furthermore, unidentified factors might impact the assessment of a mild to moderate bleeding tendency.[5] Only recently, Weisel and Litvinov[50] reported that red blood cells can influence clot permeability, suppress tPA-induced plasminogen activation, and change the fibrin network structure. Recently, we also identified blood group O, independent of VWF and factor VIII (FVIII) levels, as a risk factor for increased bleeding severity and oral mucosal bleeding in patients with BUC.[6] Seemingly paradoxically, patients with blood group O had significantly higher clot firmness. These findings suggest a VWF- and FVIII-independent mechanism linking ABO blood type and plasma clot formation, but the exact modifiers in plasma that lead to an increased bleeding risk and its potential association with the fibrinolytic system remain yet undefined.

5 | CLINICAL CONSEQUENCES AND FUTURE PERSPECTIVES

Identifying a specific diagnosis of patients with MBDs is challenging.[5] A subset of these patients have no identifiable abnormalities despite thorough and multistep laboratory workups and are classified as patients with BUC.[1] The mechanisms underlying bleeding in patients with BUC are undefined and likely differ from patient to patient. Additionally, risk factors such as the blood group may contribute to the bleeding phenotype, likely due to the multifactorial nature of risk factors for their bleeding outcome.[5,6]

The investigation of hyperfibrinolytic disorders is recommended in the second step of the diagnostic algorithm for patients with mild to moderate bleeding disorders.[1] However, reliable diagnostic assays to diagnose or screen for fibrinolytic disorders are not available.

In our opinion, a hyperfibrinolytic bleeding disorder should be suspected in patients with BUC with recurrent, abnormal posttraumatic, postsurgical, and/or mucocutaneous bleeding; a positive family history; reproductive failure; and/or bleeding in unusual sites such as intramedullary or umbilical cord bleeding.[18,21] A deficiency

in PAI-1 or α2-antiplasmin would be suspected first, as deficiencies in these factors have been established as bleeding disorders. Additional factors involved in fibrinolysis, including tPA antigen and activity and TAFI, could be investigated in patients with suspected hyperfibrinolytic bleeding. Of the available methods to investigate the overall fibrinolytic state, we suggest using fibrinolysis assays that are sensitive to plasminogen activators (eg, thromboelastometry, Table 1), as no diagnostic gold standard has been defined. In addition, we recommend measuring fibrinolytic factors with ELISA or colorimetric-based assays in selected patients from tertiary centers, in whom hyperfibrinolysis cannot be ruled out.[1,18]

We hope that future comprehensive studies assess fibrinolysis not only with global tests but also through quantification of relevant fibrinolytic factors with ELISAs or colorimetric assays. These measurements give distinct insights into alterations of the fibrinolytic system, not provided by global assays such as the ECLT, which can be insensitive to fibrinolytic factor deficiencies.[9] Although deficiencies of fibrinolytic factors are rare, measurement of these proteins is essential in the evaluation of a bleeding tendency. Additionally, as previously mentioned, age, BMI, single-nucleotide polymorphisms, and the circadian rhythm influence levels of specific fibrinolytic factors, and more comprehensive data are needed to better understand their influence in patients and healthy subjects.[9]

Hyperfibrinolysis can be treated with antifibrinolytics such as tranexamic acid (TXA) or aminocaproic acid, which are synthetic lysine analogues that block the lysine-binding sites on plasminogen, reducing the conversion of plasminogen to plasmin and preventing the interaction of plasminogen with fibrin.[51,52] Tranexamic acid is on the list of Essential Medicines of the World Health Organization[53] (Figure 1). Although it has a safe profile, TXA is given in high doses when treating severe bleedings, which is associated with more severe side effects, including seizures and hyperexcitability.[54] Aprotinin, which is a direct plasminogen inhibitor and more effective antifibrinolytic, was taken off the market due to serious adverse events, such as allergic reactions, increased mortality, and impaired renal function.[55] In recent years, new ligands of the lysine-binding site, such as piperidyl-isoxazolol derivatives and substituted pyrimidoindazolones or pyrazolopyrimidinones, were found to be more effective than TXA in preclinical studies.[55] Additionally, four inhibitors of the active site of plasminogen have been developed. However, none have made it to animal studies, most likely because of little interest from the pharmaceutical industry and the already well established and inexpensive TXA.[55]

Tranexamic acid is recommended for trauma- or surgery-induced hyperfibrinolysis and severe postpartum hemorrhage[56] and can also be used in patients with MBDs and/or BUC, for example, during menorrhagia and/or before and after hemostatic challenges such as surgery or childbirth.[1] Antifibrinolytics effectively control or prevent bleeding in patients with PAI-1 deficiency.[57]

Genetic mutations underlying a hyperfibrinolytic bleeding disorder have been identified only in patients with α2-antiplasmin deficiency (unknown prevalence, 2 mutations identified,14 homozygous cases),

PAI-1 deficiency (unknown prevalence, 3 mutations identified, 3 families), and the Quebec bleeding disorder (estimated prevalence in Quebec, Canada: 1:220 000; autosomal dominant copy number variation).[18,22-24,30,58,59] Diagnostic high-throughput sequencing including fibrinolytic genes could help identify additional fibrinolytic disorders. Nevertheless, a study by Downes et al[60] recently showed that high-throughput sequencing of coagulation-related genes only identified a significant genetic variant in 3.2% of 617 patients with BUC. In this study, patients with BUC from the VIBB cohort had no significant variants in the fibrinolytic genes *PLAT* (encoding tPA), *SERPINE1* (encoding PAI-1), *SERPINF2* (encoding α2-antiplasmin), or *PLAU* (encoding uPA) (Downes K, personal communication, June 24, 2020).[60]

6 | CONCLUSION

Detecting increased fibrinolysis is crucial in the evaluation of patients with MBDs, as hyperfibrinolysis can lead to severe bleeding and targeted treatment with antifibrinolytics is available.[9] A variety of obstacles, such as challenging techniques for measuring in vivo fibrinolysis, the absence of an easy-to-perform and widely available screening assay, and the low prevalence of congenital deficiencies of fibrinolytic factors in the literature, have likely led to an underestimation of abnormal fibrinolysis in bleeding patients.[9,18] Recent studies showed that specific biomarkers and global coagulation assays could indicate altered fibrinolytic potential in patients with mild bleeding tendencies.[34,42,47] These specific investigations, as part of the systematic approach to mild to moderate bleeding disorders, could lead to a higher diagnostic success rate in a large group of patients with undefined bleeding disorders. In the future, suitable coagulation assays and the inclusion of next-generation sequencing could improve the understanding of mechanisms underlying bleeding in patients with BUC, which may be multifactorial in a majority of affected subjects.

7 | ISTH VIRTUAL CONGRESS REPORT

A variety of abstracts covering the fibrinolytic system were presented at the ISTH 2020 Virtual Congress.[61]

With regard to genetic disorders of fibrinolytic factors, 56 patients with plasminogen deficiency from the international HISTORY registry study on patients with plasminogen deficiency were presented. These patients were identified in 26 centers in 12 countries. In total, 51 subjects, including 27 siblings with plasminogen deficiency, were asymptomatic. This example of international collaboration could be applied to alterations in other fibrinolytic factors, especially those leading to hyperfibrinolysis and bleeding, to better understand the pathophysiology and characterize phenotypes of these patients.

The physiology of fibrinolytic factors was explored in another study by Reda et al, who analyzed crosslinking between coagulation

inhibitors and fibrinolysis. They showed by comparing healthy with thrombophilic plasma (eg, plasma from patients with factor V Leiden) that activated protein C downregulates PAI-1 in vivo.

Several abstracts covered global assays that measure fibrinolysis. Dias et al analyzed the ability of different generations of viscoelastic analyzers, such as thromboelastography (TEG) and ROTEM, to detect hyperfibrinolysis. New-generation TEG/ROTEM cartridges were able to detect tPA-induced lysis, which suggests that viscoelastic hemostatic analyzer–guided treatment algorithms should be adapted to the sensitivity of the TEG/ROTEM model.

Another group around Amgalan et al presented a systematic literature review of the use of TEG/ROTEM in pregnancy and the peripartum period. In total, 63 studies, including two randomized controlled trials, with approximately 60 000 subjects were included. The authors conclude that ROTEM may identify patients at risk for severe obstetric hemorrhage, but more comprehensive randomized clinical trials are needed to validate this finding.

Several abstracts also evaluated the fibrinolytic activity in critically ill patients. In a literature review of 284 articles related to trauma-induced coagulopathy, alterations of fibrinolytic factors were found to play a crucial role in trauma-induced coagulopathy. However, the authors (Kwan et al) concluded that the mechanisms behind these alterations in fibrinolytic activity remain unclear and more data are needed to better guide the use of antifibrinolytic agents.

In addition, Rossetto et al reported that ROTEM parameters of trauma patients analyzed 24 hours after admission, rather than at hospital admission, have a higher predictive value for late clinical outcomes. Lower maximum lysis was associated with more severe traumatic brain injury and increased mortality. They also found that empiric TXA treatment reduced early mortality, by shifting patients toward a hypofibrinolytic state but did not affect late mortality.

Mertens et al found rapid generation of activated TAFI in patients with acute ischemic injury who received thrombolysis, which decreased immediately after recombinant tPA infusion. However, antigen levels of TAFI remained elevated, even after several hours, which highlights the importance of measuring both activity and antigen levels of TAFI to analyze TAFI generation in patients with acute ischemic stroke under treatment. Another study by Doyle et al studied fibrinolytic alterations during extracorporeal membrane oxygenation (ECMO). They showed in an ex vivo model that tPA antigen levels decreased and PAI-1 antigen levels increased over a 24-hour period, which suggests a possible hypofibrinolytic state in patients during ECMO.

AUTHOR CONTRIBUTIONS
All authors designed the structure of this review. DM and JG performed a literature research and wrote the manuscript. IP and CA gave input and revised the intellectual content of the manuscript. All authors approved the content of the manuscript.

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