Bleeding Disorder of Unknown Cause: A Diagnosis of Exclusion

Dino Mehic¹ Johanna Gebhart¹ Ingrid Pabinger¹

¹ Clinical Division of Haematology and Haemostaseology, Department of Medicine I, Medical University of Vienna, Vienna, Austria Address for correspondence Prof. Ingrid Pabinger, MD, Clinical Division of Hematology and Hemostaseology, Department of Medicine I, Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria (e-mail: Ingrid.pabinger@meduniwien.ac.at).

Hamostaseologie 2024;44:287-297.

Abstract

Patients with an unexplained mild to moderate bleeding tendency are diagnosed with bleeding disorder of unknown cause (BDUC), a classification reached after ruling out other mild to moderate bleeding disorders (MBD) including von Willebrand disease (VWD), platelet function defects (PFDs), coagulation factor deficiencies (CFDs), and non-hemostatic causes for bleeding. This review outlines our diagnostic approach to BDUC, a diagnosis of exclusion, drawing on current guidelines and insights from the Vienna Bleeding Biobank (VIBB). According to guidelines, we diagnose VWD based on VWF antigen and/or activity levels <50 IU/dL, with repeated VWF testing if VWF levels are <80 IU/dL. This has been introduced in our clinical routine after our findings of diagnostically relevant fluctuations of VWF levels in a high proportion of MBD patients. PFDs are identified through repeated abnormalities in light transmission aggregometry (LTA), flow cytometric mepacrine fluorescence, and glycoprotein expression analysis. Nevertheless, we experience diagnostic challenges with regard to reproducibility and unspecific alterations of LTA. For factor (F) VIII and FIX deficiency, a cutoff of 50% is utilized to ensure detection of mild hemophilia A or B. We apply established cutoffs for other rare CFD being aware that these do not clearly reflect the causal role of the bleeding tendency. Investigations into very rare bleeding disorders due to hyperfibrinolysis or increase in natural anticoagulants are limited to cases with a notable family history or distinct bleeding phenotypes considering cost-effectiveness. While the pathogenesis of BDUC remains unknown, further explorations of this intriguing area may reveal new mechanisms and therapeutic targets.

Keywords

- von Willebrand disease
- von Willebrand factor
- hemophilia A/B
- inherited platelet disorders
- hemostasis

Introduction

Mild to moderate bleeding disorders (MBDs) encompass a broad spectrum of mild bleeding symptoms, ranging from mucocutaneous bleeding, including epistaxis, easy bruising, hematomas, and heavy menstrual bleeding, to moderate (or even sever) hemorrhagic events following surgical procedures, tooth extractions, or childbirth.^{1,2} Distinguishing between clinically significant and trivial bleeding is essential before performing a comprehensive laboratory assessment.¹

received November 20, 2023 accepted after revision February 7, 2024 Bleeding assessment tools such as the ISTH-BAT or the Vicenza bleeding score can assist in the systematic assessment of the bleeding severity and documentation of all relevant bleeding symptoms.^{3,4}

In up to 60% of all patients with MBD, no underlying cause can be identified, despite exhaustive investigations of plasmatic coagulation and platelet function.^{2,5} These patients are categorized with bleeding disorder of unknown cause (BDUC).⁶ BDUC patients exhibit a bleeding phenotype and severity indistinguishable from those with well-defined mild

© 2024. Thieme. All rights reserved. Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany DOI https://doi.org/ 10.1055/a-2263-5706. ISSN 0720-9355. bleeding disorders, such as mild von Willebrand disease (VWD), mild platelet function defects (PFDs), or mild coagulation factor deficiencies (CFDs).^{7,8} Furthermore, impaired hemostatic capacity reflected by reduced thrombin generation potential and/or abnormal plasma clot formation and lysis support the diagnosis of BDUC as a separate entity of MBDs.^{9–11}

Patients with MBDs, particularly those with BDUC, face a risk of recurrent bleeding, as demonstrated by prospective data from the Vienna Bleeding Biobank (VIBB) and other research cohorts.^{12–14} In fact, a majority of BDUC patients have persistent mild bleeding symptoms, including epistaxis and easy bruising, alongside an elevated risk for more severe bleeding events following surgery, tooth extraction, or during postpartum hemorrhage (PPH). While prophylactic treatments involving desmopressin (DDAVP) and/or tranexamic acid (TXA) have shown some efficacy in reducing bleeding complications, a substantial cohort of patients continues to experience bleeding episodes.^{6,12} Moreover, these patients contend with adverse effects on their health-related quality of life, affecting both physical and mental wellbeing.¹⁵ Overall, clinical and translational data in the last decade have demonstrated the relevance of BDUC as a distinct bleeding disorder and therefore the need for identifying and registering these patients.¹⁶

In this review, we endeavor to provide a comprehensive summary of the current diagnostic protocols for MBDs within the VIBB, with the primary objective of enhancing the ability to diagnose BDUC patients accurately. We will place particular emphasis on the significance of repetitive and comprehensive testing for VWD and PFD and our diagnostic approach when investigating mild CFD.

The Vienna Bleeding Biobank: Study Cohort, Phenotype, and Diagnoses

The VIBB represents a single-center cohort study conducted at the Clinical Division of Hematology and Hemostaseology of the Medical University of Vienna.^{5,17} Patients aged 16 years and older without a previously diagnosed bleeding disorder, who are referred to our hemostasis outpatient clinic for a diagnostic work-up of their non-trivial bleeding tendency, are included based on the judgment of experienced hemostasis experts. While bleeding scores, such as the ISTH BAT or Vicenza bleeding score, are recommended for the clinical evaluation of the bleeding phenotype and are routinely conducted in all patients within the VIBB, we refrain from employing the suggested pathological cutoffs for patient inclusion. This decision is rooted in our previously published data, which revealed a low ability of bleeding scores to discriminate between BDUC and other MBDs.⁷ Additionally, bleeding scores tend to be increased with advancing age, attributable to an increased incidence of hemostatic challenges such as surgical procedures. Patients with a previous MBD diagnosis are not included. The selection process is guided by strict exclusion criteria, which encompass active pregnancy; ongoing malignancy; recent

surgical procedures within the preceding 6 weeks; bacterial infections within the last 2 weeks; continuous therapy involving anticoagulant, antiplatelet, or anti-inflammatory medications over the past 10 days; thrombocytopenia with a count below 100×10^9 /L; and impaired liver as well as kidney functions. The study was approved by the Ethics Committee of the Medical University of Vienna (EC No 603/2009), according to the Declaration of Helsinki.

Over the course of more than a decade, the VIBB has successfully enrolled an impressive cohort of over 900 patients with MBDs. The median (interquartile range) age of our patients (n = 912) is 38 (28, 52) years. Patients from the VIBB are predominantly female, which might be due to women-specific hemostatic challenges during lifetime including menstrual bleeding and childbirth. Patients with MBDs usually present with symptoms such as hematomas, easy bruising, epistaxis, and minor wound bleeding. Moreover, many individuals experience bleeding episodes following surgical procedures, tooth extractions, and PPH. Among female patients, approximately two-thirds present with heavy menstrual bleeding.^{5,8,18}

Around 64% of patients from the VIBB are classified with BDUC, followed by definite/possible PFD, VWD, and CFD (**Fig. 1**; unpublished data by Mehic et al. 2023). Within the CFD category, a majority exhibit factor (F) VIII deficiency (activity \leq 50%; n = 16; 57%), followed by FIX deficiency (activity \leq 50%; n = 8; 29%), FXI deficiency (activity \leq 60%; n = 3; 11%), and FXIII deficiency (activity \leq 10%; n = 1; 3%). The median (interquartile range) age of our patients is 38 (28,52) years. There is also a distinctive gender disparity, as 69% of all women are classified with BDUC, whereas among men, the diagnosis of BDUC stands at 50%.

Diagnostic Approach within the VIBB

A standardized and extensive diagnostic work-up to diagnose patients with BDUC has been previously described by Ross Baker and James O'Donnell as well as for MBD by Rodeghiero et al.^{1,6} Given that BDUC is a diagnosis of exclusion, the precision of evaluating other MBDs such as VWD, PFD, or CFD holds paramount importance.⁶ Despite routine diagnostic procedures and available recommendations and guidelines, certain questions remain unresolved. Among others, these include the relevance of repeated testing, the establishment of relevant cutoff values (e.g., for VWD or CFD) as well as the interpretation of abnormalities in platelet function tests of unknown clinical significance. Additionally, consideration must be given to the possibility of rare bleeding disorders attributed to hyperfibrinolysis or heightened levels of natural anticoagulants in specific patient populations.^{19,20}

Within the VIBB, our diagnostic approach combines evidence-based principles with practicality. While we adhere to current guidelines and draw from published data in literature, certain analyses, particularly for PFD, necessitate a pragmatic perspective. This approach factors in resource availability, technical constraints, and the patient's willingness for follow-up visits.



Fig. 1 Diagnoses in all patients and according to sex in the Vienna Bleeding Biobank. Previously unpublished data from the VIBB. BDUC, bleeding disorder of unknown cause; PFD, platelet function defects; VWD, von Willebrand disease; CFD, coagulation factor deficiency.

Our diagnostic criteria, depicted in **Fig. 2**, are elaborated upon in the subsequent sections, providing a comprehensive framework for our assessments.

von Willebrand Disease

When evaluating VWD, a thorough clinical and laboratory assessment is indispensable.²¹ The latest guidelines recommend the measurement of von Willebrand factor antigen (VWF:Ag), von Willebrand factor activity (VWF:Act), and factor VIII activity. To ensure precise diagnostics, functional assays like VWF:GP1bM instead of VWF:RCo assays are recommended for measuring VWF:Act.²¹⁻²³ As extensively discussed in the current VWD diagnostic guidelines,²¹ these assays exhibit lower coefficients of variations, enhanced reproducibility,^{24–27} and are unaffected by specific genetic polymorphisms.^{22,28} By applying these assessments, VWD can be categorized into three distinct subtypes.^{21,23} Accurate categorization into VWD subtypes is of importance due to variations in inheritance patterns and tailored therapeutic approaches.²³ Quantitative deficiencies manifest when VWF levels are reduced (<50 IU/dL), and the VWF:Act/VWF:Ag ratio exceeds 0.7 (VWD type 1). Patients with a severe quantitative VWF deficiency and VWF levels <3 IU/dL are classified as type 3. If the VWF:Act/VWF:Ag ratio is below 0.7, a qualitative defect (VWD type 2) is suspected.²³

The diagnosis of VWD can be complicated by factors leading to elevated VWF levels.²⁹ These confounding factors should be avoided during VWD investigations whenever

possible or considered at interpretation of results.²¹ High estrogen levels during pregnancy or hormonal contraceptives, and hormone replacement therapy, such as in the postmenopausal phase, are known to elevate VWF levels. Thus, diagnostic assessments should ideally occur during periods of normal estrogen levels (e.g., post-pregnancy). While the influence of cycle-dependent estrogen levels is considered minor, it should be taken into account when values are near the diagnostic threshold. VWF is an acutephase protein, its levels increase during inflammatory processes or trauma. In cases of suspected inflammation, measuring C-reactive protein (CRP) levels is advisable. With advancing age, VWF levels tend to rise in both healthy individuals and patients with known VWD type 1.^{30,31} An association between higher VWF and various conditions, including atherosclerosis, neoplasms, or liver diseases, is also well known.³² Physical activity, stress, or smoking can also elevate VWF levels, and these factors should also be considered in the diagnostic process.²⁹

Blood group O, on the other hand, is linked to reduced VWF levels both in healthy persons and patients with VWD type 1.^{33,34} Our research has also unveiled a VWF-independent influence of blood group O on bleeding severity in patients with BDUC.^{6,18} While determining ABO blood groups is recommended in bleeding patients,⁶ it no longer plays a role in VWD diagnosis.²¹

In general, VWF measurements have a large intra- and inter-individual variability,³⁵ and many pre-analytical



Fig. 2 Diagnostic work-up of patients with a bleeding tendency within the Vienna Bleeding Biobank. ADP, adenosine diphosphate; APTT, activated partial thromboplastin time; CD, cluster of differentiation; FV, factor V activity; FVIII, factor VIII activity; FVIX, factor IX activity; FX, factor X activity; FXI, factor XI activity; FXIII, factor XIII activity; GP, glycoprotein; PFD, platelet function defect; PT, prothrombin time; VWD, von Willebrand disease; VWF:Ag, von Willebrand factor antigen; VWF:Act, von Willebrand factor activity. ¹Including but not limited to Achenbach's syndrome, amyloidosis, angina bullosa hemorrhagica, auto-erythrocyte syndrome (also known as psychogenic purpura and Gardner-Diamond syndrome), exercise-induced purpura, hereditary hemorrhagic telangiectasia, hypothyroidism, medications, Noonan syndrome, osteogenesis imperfecta, scurvy, senile purpura, skin fragility and connective tissue disorders (e.g., Ehlers-Danlos syndrome), uremia, vasculitis (e.g., Henoch-Schönlein purpura). ¹Citrated blood was centrifuged at $150 \times g$ for 10 minutes at room temperature to produce platelet-rich plasma (PRP). ⁴CRP-XL (0.04 µg/mL, generous gift from Dr. R. W. Farndale, Department of Biochemistry, University of Cambridge, Cambridge, UK). [†]RIPA at 0.7 mg/mL to identify potential PT-VWD. ¹Euglobulin clot lysis time, levels of fibrinolytic factors (e.g., PAI-1, alpha-2-antiplasmin, tissue plasminogen activator). ¹Including but not limited to Achenbach's syndrome), exercise-induced purpura, hereditary hemorrhagic telangiectasia, hypothyroidism, medications, Noonan syndrome, osteogenesis imperfecta, scurvy, senile purpura, skin fragility and Connective tissue disorders (e.g., PAI-1, alpha-2-antiplasmin, tissue plasminogen activator). ¹Including but not limited to Achenbach's syndrome, amyloidosis, angina bullosa hemorrhagica, autoerythrocyte syndrome (also known as psychogenic purpura and Gardner-Diamond syndrome), exercise-induced purpura, hereditary hemorrhagic telangiectasia, hypothyroidis

factors including time for sample transportation as well as temperature variation may influence VWF assays.³⁶ According to current guidelines,²¹ VWD is typically ruled out when VWF:Ag and/or VWF:Act values exceed 50 IU/dL, provided that factors contributing to elevated VWF levels have been thoroughly investigated. However, in our study from the VIBB involving 277 patients with repeated VWF measurements, 13% of patients exhibited fluctuating VWF levels both below and above the 50 IU/dL diagnostic cutoff.³⁷ Notably, around 77% of patients with VWD fulfilled the diagnostic criteria at study inclusion, while 23% fulfilled the criteria at later visits. Similar patterns were observed in pediatric patients with bleeding symptoms, where VWD was initially diagnosed in only 70% of all assessed cases.³⁸ Brown et al demonstrated the need for repeated VWF measurements in adolescents with heavy menstrual bleeding, as only 70% of patients with VWD were identified during the acute bleeding situation.³⁹

In our investigation, we found that VWF levels of 80 IU/dL represented a viable threshold, with a mere 1.2% probability (95% confidence interval, 0.3–4.9) of falling below the diagnostic threshold in repeated testing.³⁷ Consequently, this threshold yielded negative predictive values of 98.1% for crossing the threshold of 50 IU/dL. These results suggest that conducting repeated VWF:Ag and VWF:Act assays only in individuals with VWF:Ag and/or VWF:Act levels below 80 IU/dL could be a valuable strategy to reduce overlap between patients with low VWF levels and those with BDUC.

Within our VIBB study, patients with platelet counts below 100×10^9 /L and possible VWD type 2B or hereditary thrombocytopenia are not included.

Our approach: For all included patients in the VIBB, we perform VWF:Ag and for VWF:Act, both VWF:GP1bM and VWF:RCo, assays as well as specific analyses (e.g., multimeric analysis, genetic testing) for subtype categorization, according to current guidelines.²¹ We also aim to perform genetic testing in all patients, regardless of subtype. This is particularly of importance for the identification of type 2N or 2B, where DDAVP treatment is contraindicated. We also acknowledge that genetic testing of all patients with VWD, who sometimes fluctuate between type 1 and type 2 VWD due to high coefficients of variations, might not be feasible in all centers. We perform ristocetin-induced platelet aggregation (RIPA) at 0.7 mg/mL to identify potential PT-VWD in addition to genetic testing of VWF, while currently RIPA mixing studies are not being performed.⁴⁰⁻⁴² Notably. PT-VWD is very rare, and no cases were identified in our exome sequencing of over 600 patients from the VIBB.43 Furthermore, recently we established the re-testing of VWF:Ag and VWF:Act levels at least once for patients with VWF levels below 80 IU/dL at study inclusion to enhance diagnostic accuracy.

Platelet Function Defects

Patients affected by PFD typically present with mucocutaneous bleeding including heavy menstrual bleeding and postsurgical bleeding or PPH.^{44–46} There is often a positive family history for a bleeding tendency and a detailed medical history with focus of potential syndromic features (e.g., facial dysmorphia, skeletal abnormalities) is essential.⁴⁷ Underlying mechanisms of PFD are remarkably diverse including dysfunctional glycoprotein receptors, defects in the signal cascade, cytoskeletal alterations, deficiencies of alpha/delta granules, and their contents as well as their release, resulting in an impaired platelet aggregation, reactivity, and granule degranulation.^{44,46–48}

PFD diagnosis poses challenges and is primarily limited to specialized centers with the necessary resources and expertise, given the need for on-site investigations of platelets and unavailability of sensitive global assays for PFD.^{44,49} While severe non-syndromal PFDs like Glanzmann thrombasthenia (deficiency of GPIIb/IIIa receptors) and Bernard-Soulier syndrome (deficiency of the GPIb–V–IX complex) can be straightforwardly identified and have been extensively studied, milder PFDs present diagnostic complexities, even for experienced centers. In the last two decades, investigations have identified other syndromes such as the Gray platelet syndrome, which is accompanied by deficiency of alpha granules.⁵⁰

The Platelet Physiology SSC of the ISTH has proposed a structured evaluation approach for suspected PFD.^{46,51} In short, light transmission aggregometry (LTA), using epinephrine, adenosine diphosphate (ADP), collagen, arachidonic acid (AA), and ristocetin as agonists, is recommended, in accordance with recent ISTH guidelines for LTA,⁵¹ assessment of platelet granule release, and the analysis of major surface glycoproteins on resting platelets as well as GPIIb/IIIa activation epitope on activated platelets by flow cytometry should also be performed, in accordance with the SSC guide-

lines. In the second step approaches, LTA with an expanded agonist panel and flow cytometry with additional antibodies for glycoprotein expression should be performed. Furthermore, the measurements of serum TxB2 (e.g., with ELISA or radioimmunoassay) and transmission electron microscopy are suggested. More specifically, absence or marked reduction of α -granules in platelets as seen in Gray platelet syndrome can be identified by electronic microscopy, which also has a better sensitivity for identifying dense granule deficiency.⁵²⁻⁵⁴ Delta storage pool disease can be investigated using a laboratory-developed mepacrine fluorescence test,^{55,56} as van Asten et al could show good discriminative power of this test.⁵⁷ However, many these recommended laboratory methods lack standardization and pose technical challenges. In cases where PFD is still suspected, biochemical and molecular genetic studies are recommended. Up to 80 genes have been suspected to be involved in PFD, but for many a definitive evidence is still lacking.^{47,58} Due to the considerable heterogeneity of PFD, often with unidentified causative genes, genetic panel testing often yields negative results.⁵⁹ Some genetic abnormalities are also associated with predisposition for developing a malignant disease such as leukemia, which must always been considered before genetic testing.⁶⁰ A comprehensive state-of-the-art review on genetic testing for PFD has recently been published by Gebetsberger et al.⁴⁷ Interestingly, high-throughput whole exome sequencing data of 96 genes, including 39 genes related to platelet disorders, in 430 MBD patients (including patients from the VIBB) revealed a genetic variant only in around 26%.43

PFA-100 is not recommended as a screening test for PFD patients,⁴⁶ due to its low prevalence of prolonged closure times in comparison to healthy controls.^{49,61–65} In line, in the VIBB, 25% of all PFD patients did not show prolonged closure times with either PFA-100 with epinephrine or with ADP.⁶⁶

In our center, we perform LTA, flow cytometric mepacrine fluorescence, and glycoprotein expression analysis (> Fig. 2), but currently are unable to measure TXB2 and to perform electron microscopy. Our experience from the VIBB highlights that in most patients with MBDs, PFD diagnosis via pathological LTA, defined by abnormal aggregation in response to distinct agonists (according to the manufacturer Bio-Data, Horsham, Pennsylvania, United States), lacks specificity, making it difficult to characterize the underlying defect. Very recently, new agonist concentrations for LTA have been proposed based on data from an international ISTH-led validation study involving 28 laboratories for the agonists ADP (2 µmol/L) and epinephrine (25 µmol/L).⁶⁷ These recommendations have not yet been implemented in our current approach (see **Fig. 2**). Another limitation is that currently we do not measure COAT platelets in our patients.⁶⁸

Furthermore, potentially pathological findings in LTA are of unknown clinical significance. Nevertheless, LTA is the gold standard for analyzing patients with MBD for potential PFD and should be offered by every center.⁴⁶ On the other hand, abnormalities of glycoprotein expression or mepacrine fluorescence are extremely rare in MBDs, based on our experience. Nonetheless, uncovering the root cause of PFD can provide valuable insights for targeted treatments.⁶⁹ While it is advisable to refer patients to hemostasis experts and specialized care centers, given that their expertise and resources markedly augment patient care and management, it is noteworthy to acknowledge that, in a substantial proportion of cases, a definitive PFD remains elusive.^{44,69}

Our approach: In the VIBB, all patients with pathologies in LTA at study inclusion, also including patients with abnormal aggregation upon stimulation with epinephrine only, are invited for a second investigation. Following this algorithm as shown in – Fig. 2, patients are categorized as patients with possible or definite PFD or revised as BDUC patient as follows:

- No pathology in the repeated LTA: re-categorized as BDUC patient.
- Reproducible pathology with 1 agonist (e.g., epinephrine): possible PFD.
- Pathology with ≥ 2 agonists: definite PFD.

Furthermore, we perform flow cytometric mepacrine fluorescence for exclusion of dense granules deficiency and glycoprotein expression analysis of CD41 (α II β integrin, GPIIb), CD61 (β 3 integrin, GPIIIa), CD42a (GPIX), CD42b (GPIb- α), CD36 (thrombospondin receptor, GPIV), and GPVI (collagen receptor); we also aim to assess activated GPIIb/IIIa and P-selectin in all patients. Albeit not recommended, we also perform PFA-100 measurements in all patients for research purposes.

Coagulation Factor Deficiencies

Deficiencies of coagulation factors are rare disorders and, if severe such as severe hemophilia, in most patients diagnosed during childhood.⁷⁰ Only patients with a less severe bleeding phenotype, such as mild hemophilia A or B or factor (F) II, V, VII, X, XI, or XIII deficiency, are often diagnosed either because one of the global tests is abnormal or because of a bleeding tendency during adolescence and adulthood.

The diagnosis of hemophilia is based on the consensus of the SSC that patients who have a FVIII or FIX levels of <40%are defined as having hemophilia. As in certain situations, and in some patients, the level could also be >40%; thus, we have decided to set our cutoff at 50% for diagnosing a FVIII or FIX deficiency. This approach is supported by Rejtő et al⁷¹ in a cohort of patients who were diagnosed with hemophilia because of a baseline FVIII level below 40%, but were later found to have higher levels. As described in Gebhart et al,⁵ in most patients with hemophilia A or B we could identify the underlying mutation in the F8 or F9 encoding gene.

Setting the cutoffs for the diagnosis of deficiency in factors II, V, VII, X, XI, and XIII is even more complicated, as there is no clear cutoff for a factor level that will lead to bleeding manifestations. Moreover, these patients are heterogeneous, and in many of those very rare clotting factor deficiencies, the clinical bleeding tendency is not associated with the coagulant activity of the various clotting factors.^{72,73}

Hypofibrinogenemia is defined at fibrinogen levels \leq 100 mg/dL. We defined patients who had levels of factors II or V

 \leq 10% and factors VII \leq 20%, respectively, as deficient.^{72,73} With regard to FXI deficiency, we set the lower margin at 60%, according to Gomez and Bolton-Maggs,⁷⁴ who observed bleeding manifestations in patients with partial FXI deficiency of up to 60% and even higher. In a most recent publication, Reitsma et al⁷⁵ found tissue factor pathway inhibitor (TFPI) as a potential modifier of bleeding risk in FXI deficiency. There is the specific experience from studies on FXI inhibitors as novel anticoagulants that even in patients with FXI levels of <10%, no increased risk of bleeding was observed.⁷⁶ With regard to FXIII deficiency, we set the cutoff at \leq 30%.⁷²

Setting cutoffs for a clinically relevant change in laboratory levels is very difficult in those rare bleeding disorders. We can never prove that a certain alteration in a laboratory value causes the bleeding tendency; in some cases this might merely be a coincidental finding, and the patient might have a BDUC. We hope that, through performing prospective observational studies and family studies, we will come closer to the clinical relevance of those rare factor deficiencies and to better identify this at risk of bleeding.

Impairment of thrombin generation in BDUC patients has been demonstrated in previous studies conducted by our team.⁹ However, the outcomes in other studies appeared inconclusive, contingent on the assay employed and the specific cohort under analysis, as outlined by Thomas et al.¹¹ It is noteworthy that Baker and O'Donnell recommend utilizing thrombin generation as a research tool rather than a means to identify BDUC patients, based on these findings.⁶

Our approach: We measure FVIII and FIX levels in all patients independent of results in global assays, in order not to miss mild deficiencies.⁷¹ For the diagnosis of FVIII and FIX deficiency, we use a cutoff of 50%, so as not to miss patients with hemophilia A or hemophilia B. Each patient with a FVIII or FIX <50% has a repeated investigation and genetic analysis of the FVIII or FIX gene. With regard to the other rare factor deficiencies, we measure only those levels based on results in prothrombin time (PT) and activated partial thromboplastin time (APPT) and use previously established cutoffs. FXIII is also measured in all patients, as its deficiency is not reflected on any global assay. We are aware that none of the predefined cutoffs indeed clearly reflects the causal role of the bleeding tendency of a patient. We repeat the determination of the factor levels and try to find the molecular defect in those patients by genetic analysis of the respective gene.

Other Rare Bleeding Disorders

In recent years, translational and genetic studies have unveiled novel bleeding disorders, shedding light on different aspects of the hemostatic system.²⁰ However, it is important to note that certain rare bleeding disorders, which do not manifest through standard global coagulation tests, may go unnoticed unless specifically investigated. These uncommon bleeding disorders, including those marked by enhanced natural anticoagulant function and hyperfibrinolysis,^{19,20} often exhibit a bleeding pattern similar to BDUC.

Thrombomodulin-associated coagulopathy arises from specific *THBD* gene variants (c.1611C > A and c.1487delCM)

293

that lead to elevated soluble TM (sTM) levels.^{77–79} These variants cause increased TM release from the endothelium into the plasma, resulting in protein C activation and subsequently bleeding, particularly after surgery or trauma, and occasionally spontaneous abdominal bleedings have been described. Interestingly, conventional coagulation tests (PT, APTT, clotting factor levels, and platelet function) remain within normal ranges, and only reduced thrombin generation was observed in these patients.^{77–79} Our analysis of sTM levels in our VIBB cohort did not reveal elevated levels in BDUC or other MBD patients, and we did not identify any link between sTM, bleeding patterns, and thrombin generation.⁸⁰

In the case of TFPI, novel B-domain variants in exon 13 of the FV-encoding gene (F5) that produce FV splice variants, causing East Texas, FV-Amsterdam, and FV-Atlanta bleeding disorders, have been described in the last two decades.⁸¹⁻⁸³ These variants activate a rarely used splice donor, leading to a truncated form of FV (FV short). FV short binds more effectively to free TFPI α , safeguarding it from degradation and cleavage, and extending its half-life. Patients with these variants experience clinical distinct bleeding symptoms, including menorrhagia, easy bruising, epistaxis, and severe bleeding post-trauma or -surgery. They also present with prolonged PT and APTT and reduced thrombin generation.^{20,81,84,85} Our study of over 600 patients from the VIBB revealed increased levels of free TFPIa in BDUC and PFD patients, correlating with abnormal thrombin generation, such as prolonged lag time and time to peak.⁸⁶ Importantly, we did not discover relevant F5 gene variants that increase FV-short,⁴³ and patients did not reach the high plasma TFPI levels observed in those with disease-causing variants.⁸⁶

Additionally, a rare bleeding disorder attributed to natural anticoagulants is α 1-antitrypsin Pittsburgh (α 1-AT-P), characterized by a Met 358 to Arg substitution in α 1-AT. This unique variant functions as a potent thrombin and contact pathway inhibitor, resulting in a severe bleeding disorder.^{87,88}

A new bleeding disorder linked to tissue factor (TF) deficiency was recently identified in a woman with unexplained bleeding.⁸⁹ This variation in the TF gene (F3) causes premature termination of protein translation, yielding a shorter TF protein (TF-short). Experiments in mice demonstrated that this variant reduces overall TF production, leading to decreased hemostatic capacity.⁸⁹

Hereditary hyperfibrinolysis is a rare condition resulting from changes in fibrinolytic factors, seen in a limited number of individuals and families.⁹⁰ Fibrinolytic bleeding disorders include α 2-antiplasmin deficiency (14 homozygous and 104 heterozygous cases), PAI-1 deficiency (26 cases), Quebec platelet disorder (23 cases), and tPA excess (4 cases), as recently reviewed by Saes et al.⁹⁰ Hyperfibrinolytic disorders are characterized by mucocutaneous bleeding and prolonged bleeding post-surgery or injury, resembling a similar bleeding profile observed in MBDs and BDUC. In hyperfibrinolytic disorders, the severity of bleeding can vary from mild to moderate, typically marked by mucocutaneous bleeding and prolonged post-surgery or injury bleeding. In some cases, the bleeding can escalate to severe and life-threatening levels, particularly in individuals with alpha-2 antiplasmin deficiency.⁹⁰ Notably, hyperfibrinolytic bleeding disorders extend beyond bleeding issues, with associations to obstetric complications such as miscarriages, particularly in patients with α 2-antiplasmin and PAI-1 deficiency.⁹⁰ Several groups have analyzed fibrinolytic factors in BDUC and other MBDs with inconclusive results, as recently summarized by our group.¹⁹ We have previously identified increased tPA activity, a paradoxical increase in thrombin-activatable fibrinolysis inhibitor and α 2-antiplasmin, with no differences in PAI-1 in 270 BDUC patients compared to 98 healthy controls.⁹¹ On the contrary, Valke et al retrospectively found abnormal ECLT and/or reduced PAI-1 antigen and activity levels in 39% of 160 BDUC patients.⁹² Overall, the frequency of fibrinolytic alterations and their clinical relevance in BDUC remains unclear due to the diagnostic challenges in assessing fibrinolysis in vitro. Novel promising assays for measuring fibrinolysis in bleeding patients have been proposed, but need to be validated in independent cohorts,^{93,94} before they can be recommended for routine use.

Identifying hyperfibrinolytic disorders is crucial because they can be effectively managed with antifibrinolytic agents like TXA or aminocaproic acid. These synthetic lysine analogs hinder the binding of lysine to plasminogen, reducing the conversion of plasminogen to plasmin and preventing its interaction with fibrin.^{19,95} Presently, the lack of standardized and sensitive global tests may lead to the under-diagnosis of hyperfibrinolysis as a cause of bleeding disorders, emphasizing the need for enhanced diagnostic tools in this field.¹⁹ On the other hand, high-throughput whole exome sequencing of fibrinolytic genes SERPINE1 (PAI-1 deficiency), SERPINF2 (alpha-2-antiplasmin deficiency), PLAT (tissue plasminogen activator), or PLAU (Quebec platelet disorder) did not identify any monogenetic fibrinolytic disorder in over 600 BDUC patients.⁴³

Within the Thrombogenomics project, high-throughput whole exome sequencing of 96 genes associated with coagulation and platelet function was conducted.⁴³ The analysis revealed pathological genetic variants only in approximately 3% of over 600 BDUC patients.

Our approach: Given the rarity of bleeding disorders associated with variations in natural anticoagulants or fibrinolytic factors, we recommend limiting their specific investigation to carefully selected cases. This primarily involves genetic sequencing, which should be reserved for patients with a remarkable family history and/or a distinct bleeding phenotype, such as a history of miscarriages in cases of fibrinolytic disorders.

Non-Hemostatic Cause for Bleeding Disorders

Bleeding tendencies can also be attributed to a diverse range of non-hemostatic disorders and conditions, and it is imperative to consider these potential causes upfront, as emphasized in a review by Thomas et al and our group.^{8,96} These conditions include various hematological disorders such as amyloidosis, which can lead to bleeding in cerebral, cutaneous, intracranial, and gastrointestinal regions.⁹⁷ Similarly, hereditary hemorrhagic telangiectasia is associated with recurrent epistaxis, gastrointestinal bleeding, and visceral arteriovenous malformations, affecting areas like the cerebral and pulmonary regions, often accompanied by telangiectasia on the skin and mucosal surfaces.⁹⁸ Congenital disorders, like connective tissue disorders, can manifest as excessive bruising,⁹⁹ and endocrinologic conditions, such as hypothyroidism, may result in a mild mucocutaneous bleeding tendency and, although rare, severe bleeding following trauma or surgery.¹⁰⁰ Numerous medications, such as antiinflammatory drugs, antibiotics, cardiovascular and lipidlowering drugs, or selective serotonin reuptake inhibitors, have the potential to adversely affect hemostasis. Consequently, they may contribute to or exacerbate hemorrhages in specific clinical scenarios.¹⁰¹ One limitation when performing laboratory investigations in MBDs is that many factors including exercise, caffeine, dark chocolate, tobacco, herbal supplements. and others can influence hemostasis.29,102-104

Our approach: Before investigating for a bleeding disorder, we exclude other underlying causes. Furthermore, we also always assess liver and kidney function to rule out liver and kidney failure as well as infection parameters (CRP and leucocyte count) to rule out an acute phase reaction when investigating for a bleeding disorder.

Conclusion

Patients with BDUC represent the majority within the spectrum of MBD patients. BDUC patients exhibit a bleeding phenotype and severity indistinguishable from other MBDs. Given that BDUC is a diagnosis of exclusion, the accurate exclusion of other MBDs and non-hemostatic causes for the bleeding tendency is essential. Within the VIBB, we perform comprehensive assessments for VWD, PFD, and CFD based on current guidelines and available resources, as detailed in this review. Nonetheless, it is crucial to acknowledge that some rare bleeding disorders may remain undiagnosed, necessitating targeted testing in patients displaying specific phenotypes.

Recent research, both within the VIBB and other studies, has brought into focus the substantial risk of recurrent bleeding in MBD and BDUC patients,¹² coupled with an overall reduction in health-related quality of life.¹⁵ Despite the administration of prophylactic hemostatic treatments before surgical procedures or childbirth, the incidence of bleeding complications remains unacceptably high. Unfortunately, our current tools do not offer the means to predict individual bleeding risk based on clinical presentation, biomarkers, or patient characteristics. The unknown pathogenesis for impaired hemostatic capacity in most BDUC patients currently hinders the development of personalized treatment options. Nevertheless, BDUC remains an exciting area of exploration, and in the age of multi-omics approaches, new mechanisms may yet be unveiled, offering potential pathways to enhance patient outcomes.

Authors' Contribution

D.M., J.G., and I.P. performed a literature review, wrote and revised the manuscript.

Funding

The Vienna Bleeding Biobank is supported by an unrestricted grant of CSL Behring, the Medical-Scientific Fund of the Mayor of the Federal Capital Vienna (grant number 20023), and the Anniversary Fund of the Austrian National Bank (grant number 18500).

Conflicts of Interest

D.M. received honoraria for advisory board meetings and lectures from CSL Behring, a research grant from CSL Behring (Prof. Heimburger award) and travel support by Sobi, Novo Nordisk, Pfizer, and Roche.

J.G. received research grants from CSL Behring, Sobi, Takeda, Amgen, and Novartis for the Medical University of Vienna; honoraria for lectures and advisory board meetings from CSL Behring, Sobi, Novartis, and Amgen. I.P. received a grant from CSL Behring for the Medical University of Vienna; honoraria from CSL Behring, Sobi, Takeda, Pfizer, for lectures and advisory board meetings; as well as travel support by Sobi.

References

- 1 Rodeghiero F, Pabinger I, Ragni M, et al. Fundamentals for a systematic approach to mild and moderate inherited bleeding disorders: an EHA consensus report. HemaSphere 2019;3(04):e286–e286
- ² Mezzano D, Quiroga T. Diagnostic challenges of inherited mild bleeding disorders: a bait for poorly explored clinical and basic research. J Thromb Haemost 2019;17(02):257–270
- 3 Rodeghiero F, Tosetto A, Abshire T, et al; ISTH/SSC Joint VWF and Perinatal/Pediatric Hemostasis Subcommittees Working Group. ISTH/SSC bleeding assessment tool: a standardized questionnaire and a proposal for a new bleeding score for inherited bleeding disorders. J Thromb Haemost 2010;8(09):2063–2065
- 4 Rodeghiero F, Castaman G, Tosetto A, et al. The discriminant power of bleeding history for the diagnosis of type 1 von Willebrand disease: an international, multicenter study. J Thromb Haemost 2005;3(12):2619–2626
- 5 Gebhart J, Hofer S, Panzer S, et al. High proportion of patients with bleeding of unknown cause in persons with a mild-tomoderate bleeding tendency: results from the Vienna Bleeding Biobank (VIBB). Haemophilia 2018;24(03):405–413
- 6 Baker RI, O'Donnell JS. How I treat bleeding disorder of unknown cause. Blood 2021;138(19):1795–1804
- 7 Gebhart J, Hofer S, Kaider A, Rejtö J, Ay C, Pabinger I. The discriminatory power of bleeding assessment tools in adult patients with a mild to moderate bleeding tendency. Eur J Intern Med 2020;78:34–40
- 8 Mehic D, Pabinger I, Gebhart J. Investigating patients for bleeding disorders when most of the "usual" ones have been ruled out. Res Pract Thromb Haemost 2023;7(08):102242
- 9 Hofer S, Ay C, Rejtö J, et al. Thrombin-generating potential, plasma clot formation, and clot lysis are impaired in patients with bleeding of unknown cause. J Thromb Haemost 2019;17 (09):1478–1488
- 10 Veen CSB, Huisman EJ, Cnossen MH, et al. Evaluation of thromboelastometry, thrombin generation and plasma clot lysis time in patients with bleeding of unknown cause: a prospective cohort study. Haemophilia 2020;26(03):e106–e115

- 11 Thomas W, White D, MacDonald S. Thrombin generation measured by two platforms in patients with a bleeding tendency: comment. J Thromb Haemost 2021;19(11):2896–2899
- 12 Mehic D, Neubauer G, Janig F, et al. Risk factors for future bleeding in patients with mild bleeding disorders: longitudinal data from the Vienna Bleeding Biobank. J Thromb Haemost 2023; 21(07):1757–1768
- 13 Veen CSB, Huisman EJ, Romano LGR, et al. Outcome of surgical interventions and deliveries in patients with bleeding of unknown cause: an observational study. Thromb Haemost 2021; 121(11):1409–1416
- 14 Castle D, Desborough MJR, Kemp M, Lowe G, Thomas W, Obaji S. Outcomes and management of pregnancy in women with bleeding disorder of unknown cause. J Thromb Haemost 2022;20(11): 2519–2525
- 15 Mehic D, Schwarz S, Shulym I, Ay C, Pabinger I, Gebhart J. Healthrelated quality of life is impaired in bleeding disorders of unknown cause: results from the Vienna Bleeding Biobank. Res Pract Thromb Haemost 2023;7(06):102176
- 16 Thomas W, Downes K, Evans G, et al. Current practice and registration patterns among United Kingdom Haemophilia Centre Doctors' Organisation centers for patients with unclassified bleeding disorders. J Thromb Haemost 2021;19(11):2738–2743
- 17 Thomas W. The natural history of bleeding disorder of unknown cause. J Thromb Haemost 2023;21(07):1747–1749
- 18 Mehic D, Hofer S, Jungbauer C, et al. Association of ABO blood group with bleeding severity in patients with bleeding of unknown cause. Blood Adv 2020;4(20):5157–5164
- 19 Mehic D, Pabinger I, Ay C, Gebhart J. Fibrinolysis and bleeding of unknown cause. Res Pract Thromb Haemost 2021;5(04):e12511
- 20 Mehic D, Colling M, Pabinger I, Gebhart J. Natural anticoagulants: a missing link in mild to moderate bleeding tendencies. Haemophilia 2021;27(05):701–709
- 21 James PD, Connell NT, Ameer B, et al. ASH ISTH NHF WFH 2021 guidelines on the diagnosis of von Willebrand disease. Blood Adv 2021;5(01):280–300
- 22 Flood VH, Gill JC, Morateck PA, et al. Common VWF exon 28 polymorphisms in African Americans affecting the VWF activity assay by ristocetin cofactor. Blood 2010;116(02):280–286
- 23 Fogarty H, Doherty D, O'Donnell JS. New developments in von Willebrand disease. Br J Haematol 2020;191(03):329–339
- 24 Boender J, Eikenboom J, van der Bom JG, et al; WiN Study Group. Clinically relevant differences between assays for von Willebrand factor activity. J Thromb Haemost 2018;16(12): 2413–2424
- 25 Costa-Pinto J, Pérez-Rodríguez A, del C Goméz-del-Castillo M, et al. Diagnosis of inherited von Willebrand disease: comparison of two methodologies and analysis of the discrepancies. Haemophilia 2014;20(04):559–567
- 26 Sagheer S, Rodgers S, Yacoub O, Dauer R, Mcrae S, Duncan E. Comparison of von Willebrand factor (VWF) activity levels determined by HemosIL AcuStar assay and HemosIL LIA assay with ristocetin cofactor assay by aggregometry. Haemophilia 2016;22(03):e200–e207
- 27 Vangenechten I, Mayger K, Smejkal P, et al. A comparative analysis of different automated von Willebrand factor glycoprotein Ib-binding activity assays in well typed von Willebrand disease patients. J Thromb Haemost 2018;16(07):1268–1277
- 28 Flood VH, Friedman KD, Gill JC, et al. No increase in bleeding identified in type 1 VWD subjects with D1472H sequence variation. Blood 2013;121(18):3742–3744
- 29 Abou-Ismail MY, James PD, Flood VH, Connell NT. Beyond the guidelines: how we approach challenging scenarios in the diagnosis and management of von Willebrand disease. J Thromb Haemost 2023;21(02):204–214
- 30 Mari D, Coppola R, Provenzano R. Hemostasis factors and aging. Exp Gerontol 2008;43(02):66–73

- 31 Sanders YV, Giezenaar MA, Laros-van Gorkom BA, et al; WiN Study Group. von Willebrand disease and aging: an evolving phenotype. J Thromb Haemost 2014;12(07):1066–1075
- 32 Atiq F, Meijer K, Eikenboom J, et al; WiN Study Group. Comorbidities associated with higher von Willebrand factor (VWF) levels may explain the age-related increase of VWF in von Willebrand disease. Br J Haematol 2018;182(01):93–105
- 33 Gill JC, Endres-Brooks J, Bauer PJ, Marks WJ Jr, Montgomery RR. The effect of ABO blood group on the diagnosis of von Willebrand disease. Blood 1987;69(06):1691–1695
- 34 Flood VH, Christopherson PA, Gill JC, et al. Clinical and laboratory variability in a cohort of patients diagnosed with type 1 VWD in the United States. Blood 2016;127(20):2481–2488
- 35 de Maat MPM, van Schie M, Kluft C, Leebeek FWG, Meijer P. Biological variation of hemostasis variables in thrombosis and bleeding: consequences for performance specifications. Clin Chem 2016;62(12):1639–1646
- 36 Preston FE, Lippi G, Favaloro EJ, Jayandharan GR, Edison ES, Srivastava A. Quality issues in laboratory haemostasis. Haemophilia 2010;16(Suppl 5):93–99
- 37 Mehic D, Kraemmer D, Tolios A, et al. The necessity of repeat testing for von Willebrand disease in adult patients with mild to moderate bleeding disorders. J Thromb Haemost 2024;22(01): 101–111
- 38 Doshi BS, Rogers RS, Whitworth HB, et al. Utility of repeat testing in the evaluation for von Willebrand disease in pediatric patients. J Thromb Haemost 2019;17(11):1838–1847
- 39 Brown MC, White MH, Friedberg R, et al. Elevated von Willebrand factor levels during heavy menstrual bleeding episodes limit the diagnostic utility for von Willebrand disease. Res Pract Thromb Haemost 2021;5(04):e12513
- 40 Soleimani R, Khourssaji M, Cabo J, et al. Letter in response to Othman & Favaloro "Comparison of two ways of performing ristocetin-induced platelet agglutination (RIPA) mixing study for diagnosis of type 2B VWD". Res Pract Thromb Haemost 2023;7 (06):102165
- 41 Favaloro EJ, Othman M. Comparison of 2 ways of performing ristocetin-induced platelet agglutination mixing study for diagnosis of type 2B von Willebrand disease. Response to the publication of Soleimani et al. Res Pract Thromb Haemost 2023;7(05):100286
- 42 Othman M, Favaloro EJ. 2B von Willebrand disease diagnosis: considerations reflecting on 2021 multisociety guidelines. Res Pract Thromb Haemost 2021;5(08):e12635
- 43 Downes K, Megy K, Duarte D, et al; NIHR BioResource. Diagnostic high-throughput sequencing of 2396 patients with bleeding, thrombotic, and platelet disorders. Blood 2019;134(23): 2082–2091
- 44 Gresele P, Bury L, Falcinelli E. Inherited platelet function disorders: algorithms for phenotypic and genetic investigation. Semin Thromb Hemost 2016;42(03):292–305
- 45 Podda G, Femia EA, Cattaneo M. Current and emerging approaches for evaluating platelet disorders. Int J Lab Hematol 2016;38(Suppl 1):50–58
- 46 Gresele PSubcommittee on Platelet Physiology of the International Society on Thrombosis and Hemostasis. Diagnosis of inherited platelet function disorders: guidance from the SSC of the ISTH. J Thromb Haemost 2015;13(02):314–322
- 47 Gebetsberger J, Mott K, Bernar A, Klopocki E, Streif W, Schulze H. State-of-the-art targeted high-throughput sequencing for detecting inherited platelet disorders. Hamostaseologie 2023; 43(04):244–251
- 48 Balduini C, Freson K, Greinacher A, et al. The EHA research roadmap: platelet disorders. HemaSphere 2021;5(07):e601
- 49 Baccolo A, Falcinelli E, Mezzasoma AM, et al. Usefulness of global tests of primary hemostasis in the initial screening of mild/moderate bleeding disorders for orienting towards von

Willebrand disease or inherited platelet functions disorders. Thromb Res 2023;221:79–82

- 50 Sims MC, Mayer L, Collins JH, et al; NIHR BioResource. Novel manifestations of immune dysregulation and granule defects in gray platelet syndrome. Blood 2020;136(17):1956–1967
- 51 Cattaneo M, Cerletti C, Harrison P, et al. Recommendations for the standardization of light transmission aggregometry: a consensus of the working party from the platelet physiology subcommittee of SSC/ISTH. J Thromb Haemost 2013;11(06):1183–1189
- 52 Gunay-Aygun M, Zivony-Elboum Y, Gumruk F, et al. Gray platelet syndrome: natural history of a large patient cohort and locus assignment to chromosome 3p. Blood 2010;116(23):4990–5001
- 53 Brunet JG, Iyer JK, Badin MS, et al. Electron microscopy examination of platelet whole mount preparations to quantitate platelet dense granule numbers: Implications for diagnosing suspected platelet function disorders due to dense granule deficiency. Int J Lab Hematol 2018;40(04):400–407
- 54 Chen D, Uhl CB, Bryant SC, et al. Diagnostic laboratory standardization and validation of platelet transmission electron microscopy. Platelets 2018;29(06):574–582
- 55 Gordon N, Thom J, Cole C, Baker R. Rapid detection of hereditary and acquired platelet storage pool deficiency by flow cytometry. Br J Haematol 1995;89(01):117–123
- 56 Wall JE, Buijs-Wilts M, Arnold JT, et al. A flow cytometric assay using mepacrine for study of uptake and release of platelet dense granule contents. Br J Haematol 1995;89(02):380–385
- 57 van Asten I, Blaauwgeers M, Granneman L, et al. Flow cytometric mepacrine fluorescence can be used for the exclusion of platelet dense granule deficiency. J Thromb Haemost 2020;18(03): 706–713
- 58 Bastida JM, Benito R, Lozano ML, et al. Molecular diagnosis of inherited coagulation and bleeding disorders. Semin Thromb Hemost 2019;45(07):695–707
- 59 Megy K, Downes K, Morel-Kopp MC, et al. GoldVariants, a resource for sharing rare genetic variants detected in bleeding, thrombotic, and platelet disorders: communication from the ISTH SSC Subcommittee on Genomics in Thrombosis and Hemostasis. J Thromb Haemost 2021;19(10):2612–2617
- 60 Downes K, Borry P, Ericson K, et al; Subcommittee on Genomics in Thrombosis, Hemostasis. Clinical management, ethics and informed consent related to multi-gene panel-based high throughput sequencing testing for platelet disorders: communication from the SSC of the ISTH. J Thromb Haemost 2020;18(10): 2751–2758
- 61 Hayward CP, Harrison P, Cattaneo M, Ortel TL, Rao AKPlatelet Physiology Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. Platelet function analyzer (PFA)-100 closure time in the evaluation of platelet disorders and platelet function. J Thromb Haemost 2006;4(02):312–319
- 62 Mezzano D, Quiroga T, Pereira J. The level of laboratory testing required for diagnosis or exclusion of a platelet function disorder using platelet aggregation and secretion assays. Semin Thromb Hemost 2009;35(02):242–254
- 63 Sladky JL, Klima J, Grooms L, Kerlin BA, O'Brien SH. The PFA-100
 ® does not predict delta-granule platelet storage pool deficiencies. Haemophilia 2012;18(04):626–629
- 64 Podda GM, Bucciarelli P, Lussana F, Lecchi A, Cattaneo M. Usefulness of PFA-100 testing in the diagnostic screening of patients with suspected abnormalities of hemostasis: comparison with the bleeding time. J Thromb Haemost 2007;5(12): 2393–2398
- 65 Kaufmann J, Adler M, Alberio L, Nagler M. Utility of the platelet function analyzer in patients with suspected platelet function disorders: diagnostic accuracy study. TH Open 2020;4(04): e427–e436

- 66 Mehic D, Rast JS, Fuchs M, et al. Utility of the platelet function analyzer (PFA-100) in patients with bleeding disorder of unknown cause. Blood 2023;142(Suppl 1):3965–3965
- 67 Alessi MC, Coxon C, Ibrahim-Kosta M, et al. Multicenter evaluation of light transmission platelet aggregation reagents: communication from the ISTH SSC Subcommittee on Platelet Physiology. J Thromb Haemost 2023;21(09):2596–2610
- 68 Segot A, Adler M, Aliotta A, et al. Low COAT platelets are frequent in patients with bleeding disorders of unknown cause (BDUC) and can be enhanced by DDAVP. J Thromb Haemost 2022;20(05): 1271–1274
- 69 Bargehr C, Knöfler R, Streif W. Treatment of inherited platelet disorders: current status and future options. Hamostaseologie 2023;43(04):261–270
- 70 Rejtő J, Reitter-Pfoertner S, Kepa S, et al. Epidemiology and treatment of patients with haemophilia in Austria update from the Austrian Haemophilia Registry. Hamostaseologie 2019;39(03):284–293
- 71 Rejtő J, Königsbrügge O, Grilz E, et al. Influence of blood group, von Willebrand factor levels, and age on factor VIII levels in nonsevere haemophilia A. J Thromb Haemost 2020;18(05): 1081–1086
- 72 Peyvandi F, Di Michele D, Bolton-Maggs PH, Lee CA, Tripodi A, Srivastava AProject on Consensus Definitions in Rare Bleeding Disorders of the Factor VIII/Factor IX Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis. Classification of rare bleeding disorders (RBDs) based on the association between coagulant factor activity and clinical bleeding severity. J Thromb Haemost 2012;10(09): 1938–1943
- 73 Palla R, Peyvandi F, Shapiro AD. Rare bleeding disorders: diagnosis and treatment. Blood 2015;125(13):2052–2061
- 74 Gomez K, Bolton-Maggs P. Factor XI deficiency. Haemophilia 2008;14(06):1183–1189
- 75 Reitsma SE, Holle LA, Bouck EG, et al. Tissue factor pathway inhibitor is a potential modifier of bleeding risk in factor XI deficiency. J Thromb Haemost 2023;21(03):467–479
- 76 Yi BA, Freedholm D, Widener N, et al. Pharmacokinetics and pharmacodynamics of abelacimab (MAA868), a novel dual inhibitor of factor XI and factor XIa. J Thromb Haemost 2022;20 (02):307–315
- 77 Dargaud Y, Scoazec JY, Wielders SJH, et al. Characterization of an autosomal dominant bleeding disorder caused by a thrombomodulin mutation. Blood 2015;125(09):1497–1501
- 78 Langdown J, Luddington RJ, Huntington JA, Baglin TP. A hereditary bleeding disorder resulting from a premature stop codon in thrombomodulin (p.Cys537Stop). Blood 2014;124(12): 1951–1956
- 79 Westbury SK, Whyte CS, Stephens J, et al; NIHR BioResource. A new pedigree with thrombomodulin-associated coagulopathy in which delayed fibrinolysis is partially attenuated by co-inherited TAFI deficiency. J Thromb Haemost 2020;18(09):2209–2214
- 80 Mehic D, Tolios A, Hofer S, et al. Thrombomodulin in patients with mild to moderate bleeding tendency. Haemophilia 2021;27 (06):1028–1036
- 81 Vincent LM, Tran S, Livaja R, Bensend TA, Milewicz DM, Dahlbäck
 B. Coagulation factor V(A2440G) causes east Texas bleeding disorder via TFPIα. J Clin Invest 2013;123(09):3777–3787
- 82 Cunha MLR, Bakhtiari K, Peter J, Marquart JA, Meijers JCM, Middeldorp S. A novel mutation in the F5 gene (factor V Amsterdam) associated with bleeding independent of factor V procoagulant function. Blood 2015;125(11):1822–1825
- 83 Zimowski KL, Ho MD, Shields JE, et al. Factor V Atlanta: a novel mutation in the F5 gene reveals potential new Cis-acting elements involved in regulating FV-short and TFPI Levels. Blood 2017;130(Suppl 1):366–366

- 84 Dahlbäck B. Novel insights into the regulation of coagulation by factor V isoforms, tissue factor pathway inhibitorα, and protein S. J Thromb Haemost 2017;15(07):1241–1250
- 85 Dahlbäck B. Pro- and anticoagulant properties of factor V in pathogenesis of thrombosis and bleeding disorders. Int J Lab Hematol 2016;38(1, Suppl 1)4–11
- 86 Mehic D, Tolios A, Hofer S, et al. Elevated levels of tissue factor pathway inhibitor in patients with mild to moderate bleeding tendency. Blood Adv 2021;5(02):391–398
- 87 Scott CF, Carrell RW, Glaser CB, Kueppers F, Lewis JH, Colman RW. Alpha-1-antitrypsin-Pittsburgh. A potent inhibitor of human plasma factor XIa, kallikrein, and factor XIIf. J Clin Invest 1986; 77(02):631–634
- 88 Owen MC, Brennan SO, Lewis JH, Carrell RW. Mutation of antitrypsin to antithrombin. Alpha 1-antitrypsin Pittsburgh (358 Met leads to Arg), a fatal bleeding disorder. N Engl J Med 1983;309(12):694–698
- 89 Schulman S, El-Darzi E, Florido MHC, et al; NIHR BioResource. A coagulation defect arising from heterozygous premature termination of tissue factor. J Clin Invest 2020;130(10):5302–5312
- 90 Saes JL, Schols SEM, van Heerde WL, Nijziel MR. Hemorrhagic disorders of fibrinolysis: a clinical review. J Thromb Haemost 2018;16(08):1498–1509
- 91 Gebhart J, Kepa S, Hofer S, et al. Fibrinolysis in patients with a mild-to-moderate bleeding tendency of unknown cause. Ann Hematol 2017;96(03):489–495
- 92 Valke LLFG, Meijer D, Nieuwenhuizen L, et al. Fibrinolytic assays in bleeding of unknown cause: improvement in diagnostic yield. Res Pract Thromb Haemost 2022;6(02):e12681
- 93 Bareille M, Hardy M, Chatelain B, Lecompte T, Mullier F. Laboratory evaluation of a new integrative assay to phenotype plasma fibrinolytic system. Thromb J 2022;20(01):73

- 94 Amiral J, Laroche M, Seghatchian J. A new assay for global fibrinolysis capacity (GFC): investigating a critical system regulating hemostasis and thrombosis and other extravascular functions. Transfus Apheresis Sci 2018;57(01):118–126
- 95 Pabinger I, Fries D, Schöchl H, Streif W, Toller W. Tranexamic acid for treatment and prophylaxis of bleeding and hyperfibrinolysis. Wien Klin Wochenschr 2017;129(9-10):303–316
- 96 Thomas W, Downes K, Desborough MJR. Bleeding of unknown cause and unclassified bleeding disorders; diagnosis, pathophysiology and management. Haemophilia 2020;26(06):946–957
- 97 Sucker C, Hetzel GR, Grabensee B, Stockschlaeder M, Scharf RE. Amyloidosis and bleeding: pathophysiology, diagnosis, and therapy. Am J Kidney Dis 2006;47(06):947–955
- 98 Kritharis A, Al-Samkari H, Kuter DJ. Hereditary hemorrhagic telangiectasia: diagnosis and management from the hematologist's perspective. Haematologica 2018;103(09):1433–1443
- 99 Kumskova M, Flora GD, Staber J, Lentz SR, Chauhan AK. Characterization of bleeding symptoms in Ehlers-Danlos syndrome. J Thromb Haemost 2023;21(07):1824–1830
- 100 Franchini M. Hemostatic changes in thyroid diseases: haemostasis and thrombosis. Hematology 2006;11(03):203–208
- 101 Scharf RE. Drugs that affect platelet function. Semin Thromb Hemost 2012;38(08):865–883
- 102 Rebecca Couris R. Vitamins and minerals that affect hemostasis and antithrombotic therapies. Thromb Res 2005;117(1-2):25–31, discussion 39–42
- 103 FitzGerald GA, Oates JA, Nowak J. Cigarette smoking and hemostatic function. Am Heart J 1988;115(1, Pt 2):267–271
- 104 Montagnana M, Danese E, Angelino D, et al. Dark chocolate modulates platelet function with a mechanism mediated by Flavan-3-Ol metabolites. Medicine (Baltimore) 2018;97(49): e13432

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited