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REVIEW

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How to investigate mild to moderate bleeding disorders and bleeding disorder of unknown cause

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Abstract

A bleeding tendency is one of the most common complaints observed by hematologists. It is challenging to differentiate a clinically insignificant bleeding from a bleeding phenotype that requires hemostatic evaluation and medical intervention. A thorough review of personal and familial history, objective assessment of bleeding severity using a bleeding assessment tool, and a focused physical examination are critical to correctly identifying suspected patients with mild to moderate bleeding disorders (MBDs). A basic laboratory work-up should be performed in all patients referred for a bleeding tendency. If a hemostatic abnormality is found such as evidence of von Willebrand disease, a platelet function disorder, or a coagulation factor deficiency, more extensive testing should be performed to further characterize the bleeding disorder. Conversely, if all results are normal the patient is considered to have bleeding disorder of unknown cause (BDUC). For patients with BDUC, further evaluation may include non-routine testing to look for rare bleeding disorders not detected by routine hemostasis tests, such as thrombomodulin-associated coagulopathy, tissue factor pathway inhibitor-related bleeding disorder, hyperfibrinolytic-bleeding disorders or impaired tissue factor production. In this review, we summarize the stepwise diagnostic procedure in MBDs and provide some insights into the biological features of BDUC.

KEYWORDS

bleeding disorder of unknown cause, coagulation factor deficiency, hemostasis, mild bleeding disorders, platelet function disorders

1 | INTRODUCTION

Evaluating a patient suspected of having a mild to moderate bleeding disorder (MBD) is one of the more challenging tasks in hematology. Many features of the bleeding history are subjective, distinguishing nuisance bleeding from pathologic bleeding is difficult, family history is often inconclusive, and extensive diagnostic testing is often normal.[1,2]

Recently, an International Working Group established by the European Hematology Association proposed several definitions to better categorize patients with MBD and to distinguish true bleeders from subjects with some trivial bleeding manifestation but otherwise not qualifying for being affected by a true bleeding disorder.[3] Thus, a MBD is defined as excessive bleeding characterized by frequent or intermittent bleeding symptoms that prompt hemostatic investigations, education on medications and lifestyle, and may require specific

medical interventions.[3] Several studies have shown that only about 30% of patients with MBDs are finally diagnosed with an established bleeding disorder, such as von Willebrand disease (VWD), mild to moderate coagulation factor deficiencies (CFDs) or platelet function disorders (PFD).[4–6] The remaining 70% of patients with MBD currently receive a diagnosis of bleeding disorder of unknown cause (BDUC), a newly recognized entity in recent years.[7] In the large cohort of patients included in the Vienna Bleeding Biobank ($n = 418$), 56 (13.4%) patients were diagnosed with PFD or probable PFD, 37 (8.8%) with VWD, 18 (4.3%) with clotting factor deficiencies and one (0.2%) with dysfibrinogenemia. All other patients ($n = 303$, 72.5%) had normal results in coagulation assays and were categorized as patients with BDUC.[4]

A multi-step diagnostic approach is typically employed in patients with MBDs, with minor differences among centers based on local practices.[8] The first clinical step is to obtain and analyze a personal and family history of bleeding. Subsequently, the bleeding phenotype is objectively evaluated using a validated bleeding assessment tool (BAT) followed by a focused physical examination.[9,10] The second step in the diagnosis of MBDs involves a series of laboratory tests to identify any underlying primary hemostasis or coagulation anomaly.[3] If no hemostatic abnormalities are found following these investigations, the patient is diagnosed with BDUC.[4] In this review, we will examine each facet of the multi-step diagnosis algorithm in MBD and provide insights on the distinctive biological characteristics of BDUC.

1.1 | Personal bleeding history

Patients referred for investigation of a MBD should undergo a thorough evaluation of their personal and family bleeding history.[10] A precise evaluation of both personal and family bleeding history is crucial in accurately diagnosing MBD. This evaluation of the bleeding history and pattern of bleeding can aid in determining whether the patient suffers from a primary hemostasis or a coagulation defect and whether the bleeding disorder is inherited or acquired (Table 1). Liver disease, hypothyroidism, amyloidosis, senile purpura, Cushing's syndrome, scurvy, and drugs or herbal preparation intake (e.g., molecules affecting platelets functions or causing vitamin K deficiency) are common causes of an acquired bleeding tendency and should be ruled out.[11] Similarly, hematologic disorders can be associated with acquired coagulation factor deficiencies or PFD.[11] During the review, it is important to record any previous bleeding events during infancy and childhood (although not common in MBD), including bleeding from the umbilical stump, bleeding at circumcision, cheek hematoma caused by sucking, and bleeding after the loss of deciduous teeth. However, symptoms of MBD occur more frequently during adolescence and adulthood as individuals are more frequently exposed to situations with higher bleeding risk, such as surgeries or deliveries, and menstrual bleeding in women.[5] Heavy menstrual bleeding (HMB) is a very common clinical manifestation in women with MBD and often the presenting symptom. In the recent data from the Rare Bleeding Disorders in the Netherlands (RBiN) study (also including some patients with severe factor deficiencies not fitting the definition of MBD), 80% (89/111) of women suffered from HMB.[12] In the large cohort of patients with MBD from the Vienna bleeding biobank, HMB was reported by almost 64.5% of women with MBD, and over 69.0% of women with BDUC.[13] Characteristics of HMB that indicate an underlying MBD include abnormal bleeding since menarche, HMB that requires medical intervention or is nonresponsive to treatment, recurrent ovulation bleeding, iron-deficiency or iron-deficiency anemia, a sensation of flooding, bleeding through sanitary protection within 2 h, and a menstrual period lasting more than 7 days.[14] The Vienna Bleeding Biobank indicated a significant incidence of postpartum hemorrhage in women with MBD, as reported by 34.2% of women who gave birth overall, and 31.9% of women with BDUC, in line with the RBiN study.[13,15] Obstetrical history should include the type of delivery, the modality of anesthesia and the hemostatic treatment received.[16] To document bleeding outcomes following hemostatic challenges in the context of MBD, inquiries must specifically address the type of surgery, the bleeding character, procedure/anesthesia time, use of hemostatic interventions, and wound healing.

TABLE 1 Some clues to distinguish between an inherited or acquired mild bleeding disorder.

Inherited	Acquired
Early onset bleeding, sometime in infancy and childhood	Late onset of symptoms, without history of bleeding in infancy and childhood
No comorbidities associated with bleeding	Comorbidities associated with bleeding
History of surgeries complicated with bleeding	Uneventful surgery before onset of bleeding, such as dental extractions
Family history of bleeding	Negative family history
Heavy menstrual bleeding from menarche and iron deficiency	Normal menstrual bleeding before the onset of the bleeding tendency
Symptoms worsened by intake of drugs affecting platelets functions	Recent introduction of drugs or herbal preparations affecting the platelet function or coagulation factors[a]

[a]For example, anticoagulant, antiplatelets, non-steroidal anti-inflammatory drugs, antibiotics, selective serotonin reuptake inhibitors, vitamin E, gingko biloba, and ginger.

1.2 | Bleeding assessment tool

The utilization of a standardized bleeding assessment tool (BAT) is essential to evaluate a bleeding tendency.[17] The International Society on Thrombosis and Haemostasis (ISTH) BAT is commonly used.[18] The normal range is ≤3 in men, ≤5 in women and ≤2 in children. Recently, it has been reported that the ISTH BAT normal ranges vary with age in adult healthy females. Thus, adjusting the ISTH BAT cut-off to consider age could enhance the accuracy of bleeding evaluation in women.[19]

Although the ISTH BAT was originally developed and validated for screening of suspected VWD,[20] it has been investigated in other clinical settings including MBD, VWD, BDUC, and IPFD populations.[9,17,21–23] Nevertheless, the bleeding score could not distinguish between patients with BDUC and other MBD diagnoses.[24] In a large study including 359 patients (228 with BDUC, 64%) from the Vienna Bleeding Biobank, the ISTH BAT had a low sensitivity and specificity for the diagnosis of a bleeding disorder. A sub-group evaluation of different bleeding symptoms revealed that postpartum bleeding and bleeding from small wounds were predictive for diagnosing a MBD in multivariable analysis.[23] Besides its screening purpose, a higher ISTH-BAT can have a predictive role, as it has been associated with an increased risk of future bleeding in PFD, VWD, factor XIII deficiency, and MBD.[21,25–27] Another major benefit of a significantly higher ISTH BAT score may be to limit extensive laboratory testing to patients with a higher pre-test probability of being true bleeders, thereby excluding most false-positive subjects with trivial bleeds that are common even in normal populations. However, in MBD patients seen in a tertiary care center, there was no difference in the bleeding score between patients with BDUC and established MBD diagnoses.[13] Thus, current data do not support restricting diagnostic testing to patients with a higher bleeding score. Finally, ISTH BAT allows for more reproducible history taking within the same hospital clinical team, as well as standardizing the bleeding phenotype in collaborative studies.[28]

1.3 | Physical examination

The focus of the physical examination is the identification of findings that may be indicative of an underlying diagnosis. The pattern, age and distribution of bruises may point out self-inflicted injuries, non-accidental episodes, or trauma.[10] The presence of petechiae can indicate thrombocytopenia. Mucocutaneous telangiectasis around lips or on the fingertips are commonly reported in hereditary hemorrhagic telangiectasia. Perifollicular hemorrhage and gingival bleeding are observed in scurvy. Splenomegaly may suggest a liver disease or a hematologic disease. Joint hypermobility associated with skin hyperextension are peculiar manifestations of Ehlers-Danlos syndrome. Peri-orbital purpura and macroglossia are typical of amyloidosis. Syndromic features such as hearing loss, face, or bone dysmorphisms, skin discoloration, ocular involvement are suggestive of an underlying inherited PFD.[29]

1.4 | Laboratory assessments in patients with MBDs

A basic assessment should be performed in every patient with suspected MBD. Sample collection, transport, preparation, storage, and processing should be performed by experienced coagulation technologists and laboratories according to international recommendations.[30,31] The basic assessment includes a complete blood count and examination of the peripheral blood smear, prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen activity. In patients with a high probability of MBD (based on the personal and family history, physical examination, and BAT), the baseline assessment should also include assessment of platelet function by light transmission aggregometry (LTA), measurement of VWF antigen and activity, and activities of the coagulation factors VIII and IX, as mild deficiencies may be missed by the APTT (Figure 1).[3] The full blood count and the examination of the blood smear should screen for thrombocytopenia, macrothrombocytopenia and morphological abnormalities (e.g., presence of Dohle bodies in MYH9-related disorders or the lack of platelet granule staining in gray platelet syndrome).[29] Light platelet aggregometry is valuable in detecting PFD in patients referred for a MBD, especially when it is abnormal to multiple agonists.[32] Abnormal LTA requires careful interpretation to determine whether the observed pattern reflects Glanzmann thrombasthenia, Bernard Soulier Syndrome, aspirin-like defect, P2Y12 defect, platelet-type VWD, type 2B VWD, or defects in other platelet signaling pathways.[33] Assessment of platelet granule release, expression of major platelet surface glycoproteins by flow cytometry, genetic testing and electron microscopy are further tests to characterize PFD, which mostly are restricted to specialized laboratories.[34–36] As an example, dense granule deficiency may not be detected by platelet light transmission aggregometry or lumiaggregometry and could require assessment by whole mount electron microscopy.[37] When interpreting factor VIII, VWF antigen and activity, and fibrinogen levels, it is important to keep in mind that they can be increased by exercise, stress, acute infection or inflammation, malignancy, previous surgery and pregnancy, and other clinical factors. Thus, it may be necessary to conduct repeated VWF testing for an accurate diagnosis of VWD in patients with MBD.[38] It is beyond the scope of this review to provide details of the complete investigations for each specific disease; we refer to guidelines and recommendations for specific evidence-based strategies, in particular for PFD,[29] VWD,[39] fibrinogen disorders,[40] and deficiencies of factor VIII and factor IX,[41] factor XI,[42] and factor XIII.[43]

If all results are normal, thus the patient is considered to have BDUC. As previously mentioned, BDUC can be distinguished neither by the pattern of the bleeding phenotype nor by the BAT. Therefore, the diagnosis is determined on the accuracy of excluded diagnoses.

1.5 | Global assays in patients with MBDs and BDUC

Global assays have been applied with the aim to depict the overall hemostatic capacity of patients with hemostatic disorders. The skin bleeding time (BT) assays evaluated primary hemostasis, considering its interactions with plasmatic coagulation and fibrinolysis and has been found to be pathological in 27% of MBD patients, and the only pathological finding in 19% of BDUC patients.[44] As an invasive, non-objective poorly standardized and time-consuming test, the skin BT is not currently recommended for use in the evaluation of bleeding patients. The skin BT was further developed to the PFA-100/200, a

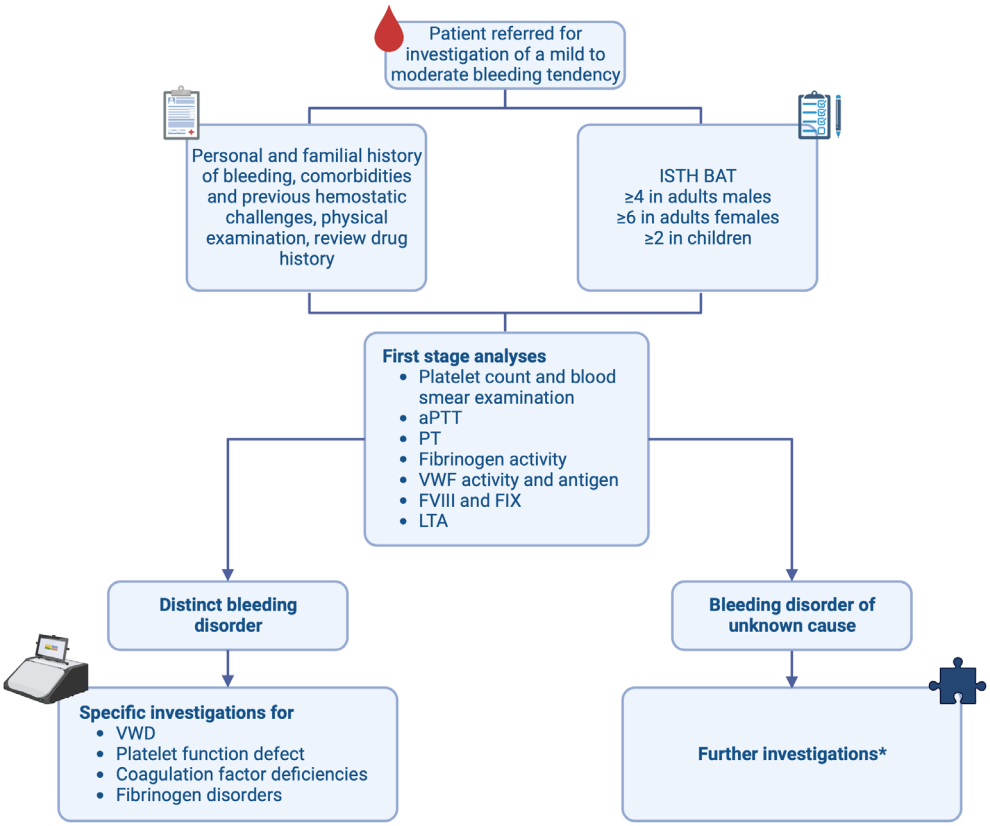

FIGURE 1 Multistep diagnostic approach for patients referred for a mild to moderate bleeding disorder (MBD). *Additional research investigations and collaborative studies can be considered for bleeding disorders of unknown cause, including for instance studies of fibrinolysis, vascular disorders, abnormalities in natural anticoagulant pathway, COAT platelets.

non-invasive in vitro assay to determine primary hemostatic defects, especially in PFD and VWD. Full data on BDUC patients are yet not available.

Additionally, there has been a recent surge of interest in studying thrombin generation in both whole blood and plasma, as it represents the endogenous potential of plasmatic coagulation that leads to fibrin production. Besides VWD and CFD patients, thrombin generation has also been the most widely investigated global assay in BDUC patients, as comprehensively reviewed by Thomas et al.[45] As a mechanism of impaired thrombin generation, defective generation of procoagulant, collagen and thrombin activated (COAT), coated platelets has been explored as a potential cause of bleeding tendency in BDUC patients. These platelets are generated after combined activation by collagen plus thrombin, are coated with alpha-granule proteins retained on their surface by a serotonin-transglutaminase-dependent mechanism and are highly efficient in maintaining thrombin generation. In a recent study investigating three cohorts of BDUC patients, Segot et al. reported a reduced ability to form COAT platelets in 36 of 157 patients with BDUC (23%).[46]

Turbidimetric assays of plasma clot formation and lysis have been examined in bleeding patients to assess possible anomalies in fibrin clot formation and structure.[47] Conversely, the euglobulin clot lysis time (ECTL) and fluorescent plasmin generation (PG) assays have been employed to elucidate the complex function of fibrinolysis in bleeding conditions. Viscoelastic assays, encompassing fibrinolysis, have become routine for point-of-care testing in trauma patients and surgical procedures. Recent research utilizing flow chambers and microfluidics has further investigated the complexities of blood flow dynamics and vascular injury. Despite these increasing developments and efforts to develop reproducible global tests to assess the hemostatic and fibrinolytic capacity in MBD patients, their clinical use is currently not recommended.[3]

1.6 | Investigation of rare bleeding disorders in patients with MBDs and BDUC

The diagnostic process to identify BDUC encompasses standard bleeding disorders like VWD, PFD, and CFDs. Nevertheless, uncommon bleeding disorders that elude detection via routine global coagulation tests might remain undiagnosed unless directly evaluated, as, for instance, PFDs with an impaired ability to support coagulation by changes in phospholipid membranes. The most severe is the Scott Syndrome, which result from pathogenic variants in *ANO6* coding for a calcium-activated ion channel responsible for the redistribution of negatively charged phosphatidyl serine and phosphatidyl ethanolamine from the platelet membrane leaflet to the outer leaflet.[48] The diagnosis is based on elevated residual levels of prothrombin in sera and defect of platelet procoagulant activity. Full diagnosis requires flow cytometry measurements of platelet phosphatidylserine exposure upon activation by thrombin and collagen.[48]

Other rare disorders involve disorders associated with natural anticoagulants and hyperfibrinolytic disorders.[49,50] Pathogenic mutations in two natural anticoagulation pathways, namely

thrombomodulin (TM)[51–53] and tissue factor pathway inhibitor (TFPI),[54–56] have been identified in individual patients or families as the cause of bleeding tendency.[50]

In TM associated coagulopathy, heterozygous variants in the *THBD* gene (c.1611C > A and c.1487delCM) lead to loss of the C-terminal cytoplasmic domain and consequent shedding of TM from the endothelium into the plasma. Patients predominantly experience postoperative and post-traumatic bleeding, although some cases also report spontaneous abdominal bleeding. The coagulation screening tests, including APTT and PT, coagulation factor levels, and platelet function tests, were normal in the patients, however, thrombin generation was reduced in these individuals. Conversely, a study of a large cohort of MBD patients, including mostly BDUC patients from the Vienna Bleeding Biobank, showed no elevated sTM levels. Additionally, there was no observed association between sTM and the bleeding phenotype or thrombin generation in patients with MBD and/or BDUC.[57]

Furthermore, novel B-domain variants in exon 13 of the *F5* gene encoding Factor V have been identified as the cause of TFPI-related bleeding disorders, specifically East Texas, FV-Amsterdam, and FV-Atlanta. These variants activate a rarely used splice donor site, resulting in truncated Factor V (FV-short), which prolongs the half-life of free TFPIα by increasing its binding and protection from degradation and cleavage. Along with clinical bleeding symptoms like menorrhagia, bruising, epistaxis, and massive bleeding following surgery or trauma, patients frequently demonstrate prolonged APTT and PT, as well as reduced thrombin generation.[50,58,59] Free TFPIα levels were evaluated in more than 600 patients with MBD from the Vienna Bleeding Biobank. Elevated free TFPIα levels were identified in patients with BDUC or PFD and were associated with irregular and delayed thrombin generation.[60] No relevant variants that would lead to an increase in FV-short were found in the *F5* gene in this study.

Another bleeding disorder related to increased endogenous anticoagulant activity is α1-antitrypsin Pittsburgh (α1-AT-P), which is a strong inhibitor of thrombin and arginine-specific enzymes in the contact system of plasma proteolysis (which includes factor XIa, kallikrein, and factor XII). Clinically this rare disorder leads to a severe bleeding phenotype with various hemorrhagic manifestations.[61,62]

Hyperfibrinolytic bleeding disorders often manifest with mucosal bleeding, and prolonged bleeding after surgery or trauma, symptoms which are also seen with MBD. Genetically determined hyperfibrinolytic disorders are rare and have only been identified in a handful of patients and families. These disorders include α2-antiplasmin deficiency (homozygous: 14 cases; heterozygous 104 cases), PAI-1 deficiency (26 cases), Quebec platelet disorder (23 cases), and excess of tPA ($n = 4$).[49]

Recently, a novel bleeding disorder due to impaired tissue factor (TF) production has been identified in a woman with a bleeding tendency of unknown cause. In detail, a *F3* gene mutation results in premature termination of protein translation and thus production of a shortened TF protein (TF short). This modification underlies a reduced hemostatic capacity as suggested by experimental evidence in mice.[63]

In general, genetic screening is not feasible to diagnose rare coagulation disorders in BDUC patients, as pathological genetic variants were only identified in approximately 3% of over 600 patients with BDUC in high-throughput whole exome sequencing of 96 genes of coagulation and platelet function within the Thrombogenomics Project.[64] Thus, the identification of these rare bleeding disorders is restricted to specialized laboratories and limited to selected MBD patients with a typical bleeding phenotype and a positive family history.

2 | CONCLUSIONS

MBD and BDUC encompass a wide spectrum of hemostatic abnormalities. An accurate stepwise approach is necessary to avoid missing the more common VWD, PFD and CFDs. BDUC is a diagnosis of exclusion after extensive laboratory testing. While research in this field has already led to a better understanding of the concepts of coagulation mechanisms, we anticipate that further discoveries in the near future will help to elucidate many other aspects of the puzzling bleeding phenotype in BDUC patients.

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DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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