BLEEDING DISORDERS

Diagnostic approaches to bleeding disorders

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Introduction and general approach

The evaluation of a patient with a possible bleeding disorder can be one of the most challenging referrals in hematology practice. In the absence of much evidence-based outcomes data, the practitioner’s experimental judgment will be called upon to formulate a working diagnosis and management plan in many instances. A logical and semi-standardized approach should include the application of a three-part screening process, each of which addresses a specific question, as follows:

1. A comprehensive bleeding history should be obtained with the specific question; ‘what is the pre-test probability that this patient has a bleeding disorder?’ One possible outcome is that no further laboratory evaluation is deemed to be necessary, for example in a patient who complains of minimal mucocutaneous bleeding symptoms, and who has had many hemostatic challenges without excess bleeding.

2. Global screening tests of hemostasis should then be applied with the specific question; ‘what is the likelihood that there is a hemostatic abnormality to explain (and are the screening tests compatible with) the patient’s history?’ Again, depending on the perceived pre-test probability and the result of these screening test(s), no further evaluation may be judged to be necessary.

3. Specific screening tests of hemostasis may then be applied in order to address the question; ‘what specific hemostatic disorder does this patient suffer from?’

Unfortunately, the available global and specific laboratory assays suffer from many limitations, including sensitivity, specificity, reproducibility, and inconsistency in interpretation between laboratories. Given all these limitations, it is not uncommon to encounter patients with a history that is strongly suggestive of a bleeding disorder yet no diagnosis is established after an exhaustive laboratory evaluation. Because it is generally not feasible to refer every such patient to a research laboratory for advanced investigation, empiric management plans may need to be instituted, using some combination of available hemostatic therapies.

The bleeding history: The best screening test for a bleeding disorder?

The bleeding history should include the age of onset of symptomatic bleeding, the pattern of bleeding both with respect to anatomical distribution (mucocutaneous vs. deep tissue) and time of onset after hemostatic challenges, and whether hemorrhage has been spontaneous and/or provoked after minor and major hemostatic challenges. A detailed family pedigree, and inquiry about systemic disorders and medication exposure are also important historical aspects. The details of excessive site-specific bleeding should be carefully documented. For example, historical features of menorrhagia that are predictive of an underlying bleeding disorder are an onset at menarche, concomitant iron deficiency anemia, hourly or greater pad/tampon changes, and the passage of clots. Until recently, there have been few published studies on the positive predictive value of site-specific or global bleeding symptoms. Indeed, relatively sparse attention has been paid to the development and validation of user-friendly bleeding scales that are also highly predictive of an underlying disorder.
Possible exceptions are the menstrual blood loss (MBL) scale developed by Kadir et al., although the definition of ‘abnormal’ (>80 mL of blood loss/period) is measured by the alkaline hematin method which is not readily performed in a clinical practice setting. A pictorial chart illustrating blood loss on sanitary napkins with an associated scoring system is more practical, and has been reasonably well validated. A pictorial blood assessment score >100 is frequently used as cut-off to define excessive menorrhagia.

In the ISTH criteria for the diagnosis of von Willebrand disease, a positive muco-cutaneous bleeding history is defined by ≥2 symptoms in the absence of a blood transfusion history, or one symptom requiring blood transfusion, or one symptom recurring on ≥3 distinct occasions. The ongoing European Union collaborative study on type 1 von Willebrand disease (MCMDM-1VWD) has recently developed and validated a questionnaire for muco-cutaneous bleeding that ascribes a severity score based on the clinical impact/outcome of site-specific bleeding; because it is being correlated with laboratory parameters and vWF genotype, it may become the standard assessment tool in the future.

What are the best laboratory global screening tests for a bleeding disorder?

Global screening tests of hemostasis should evaluate for both ‘primary’ and ‘secondary’ hemostatic abnormalities. The Prothrombin and Activated Partial Thromboplastin Times (PT & APTT) are reasonably sensitive and specific assays for factor deficiency states. Furthermore, these tests are reproducible, inexpensive, and easily performed. A platelet count and review of platelet morphology by simple light microscopy (which may pick up abnormalities such as Gray Platelet and Wiskott-Aldrich Syndromes) is mandatory.

More uncertainty revolves around the best laboratory screening strategy for vWD or platelet function abnormalities in patients presenting with a history of abnormal muco-cutaneous bleeding. Formerly, the Bleeding Time (BT) was most commonly used for this purpose, but it is invasive, relatively insensitive, and poorly reproducible. Many laboratories have now either abandoned the BT completely or added the Platelet Function Analyzer (PFA-100™) as a global screen for platelet disorders. The PFA-100 is sensitive to abnormalities that affect high shear-dependent platelet adhesion and aggregation; its utility is significantly better than the BT in the detection of vWD and severe platelet function defects (e.g., Glanzmann’s Thrombasthenia, Bernard-Soulier Syndrome), but like the BT, it suffers from a lack of sensitivity in the detection of mild platelet function disorders, such as storage pool defects and platelet secretion defects. This is an unfortunate weakness as these disorders are relatively prevalent.

In practice, many hematologists elect to screen for vWD in patients with a compatible muco-cutaneous and/or post-surgical bleeding history, and reserve referral for platelet aggregation studies (to evaluate for intrinsic platelet disorders) for those subjects in whom vWD has been ruled out. The PFA-100 pattern of abnormality may be particularly useful in this regard; when both the collagen/epinephrine and collagen/ADP closure times are abnormally prolonged, vWD is more likely. Conversely, when just the collagen/epinephrine closure time is prolonged—and salicylate/NSAID ingestion has been ruled out—platelet aggregrometry is more likely to reveal an abnormality of platelet function. A normal PFA-100 study has a reasonably high negative predictive value for vWD, but probably not for intrinsic platelet function defects. Therefore, platelet aggregrometry should be considered in any patient with a suggestive history but normal screening studies.

Testing for von Willebrand Disease (vWD)

Testing for vWD using a combination of the factor VIII activity assay (FVIII:C), vW antigen (VW:Ag) and ristocetin cofactor (VW:RCo) activities is indicated in most patients presenting with muco-cutaneous bleeding manifestations. Sub-type classification by analysis of vW multimers can be reserved for patients with suggestive abnormalities on the above screen. Once again, the historical details will provide some measure of pre-test probability of vWD. For example, it has been estimated that 5–20% of women with menorrhagia have underlying vWD. Conversely, 60–95% of women with vWD suffer from menorrhagia. It should be borne in mind that the diagnosis of vWD may be masked by the fact that assays for the vW factor complex may be erroneously high during periods of inflammation and ‘stress’ (e.g., in young children subjected to phlebotomy and in many hospitalized patients) and perhaps in women on estrogen therapy. In women of menstruating age, most studies agree that testing should be performed on days 1–4 of the menstrual cycle, when vW complex proteins may be at their nadir.

Type 1, characterized by a partial quantitative deficiency of vWF, is the most common variant, accounting for 70–80% of all vWD. It has pointed out that the application of commonly used diagnostic criteria for type 1 vWD may include many false positives, since bleeding and low vWF levels often associate by chance. Notwithstanding this proviso, type 1 vWD is defined by a quantitative deficiency of vWF – that is, vW:Ag and vWF:RCo levels >2 S.D. below the (ABO blood type population-adjusted) mean, with a normal vW multimer pattern – in a patient with bleeding symptoms and a positive family
history. Any diagnostic algorithm is further complicated by the fact that the disorder, although classically thought of as autosomal dominant, is variably penetrant in many families. Among the causes of acquired vWD, hypothyroidism is probably the most prevalent, and a routine screen of TSH levels as part of a bleeding work-up is appropriate. A FVIII/vWF:Ag ratio ≥ 1.50 (compared to ≤ 1.10 in healthy subjects) is helpful in the diagnosis of type 1 vWD, although vWF:RCo levels are the single measure of vWF-related activities that best correlate with bleeding severity. Recent data from the on-going MCMDM 1-VWD study suggest that ≈70% of subjects diagnosed with type 1 vWD have a detectable candidate mutation in the vWF gene, the majority of which are missense mutations.

Platelet aggregation tests

Although still considered the ‘gold standard’ for the diagnosis of intrinsic platelet function defects, platelet aggregation studies, which were first introduced in the 1960s, suffer from poor accuracy and reproducibility. Aggregometry is usually performed using ADP, epinephrine, arachidonic acid, collagen, and ristocetin agonists. Patients undergoing platelet function tests (PFTs) should be instructed to refrain from ingestion of all potentially platelet inhibitory drugs for at least 10 days prior to testing. Dietary factors may also affect aggregation responses. Recent studies have begun to re-address some of the standardization challenges of these assays, including pre-analytical variables. In particular, it has been suggested that the addition of platelet-poor plasma (PPP) to platelet-rich plasma (PRP) to adjust the cell count may inhibit aggregation responses, and it may be preferable to simply use unadjusted PRP; however, this is not yet considered to be standard practice. The variability in clinical laboratory practice in the execution of platelet aggregometry (concentration of agonists, use of reference ranges, mode of reporting, etc) is highlighted by a recent survey that included 47 US coagulation laboratories. Some of the variability is explained by a lack of standardized protocols. However, even when provided with a standardized methodology, wide variability still exists among platelet function studies performed at different sites. It is hoped that the pre-analytical and assay variables in platelet aggregometry can be better understood and standardized in the future as a result of recent re-evaluation.

Standard platelet aggregometry is not sensitive to secretion or storage defects, and indeed in classic delta storage pool deficiency (δ-SPD), it is well recognized that platelet aggregation may be normal. Therefore it is recommended that lumi-aggregometry (which evaluates for simultaneous ATP release in response to agonists such as thrombin and ADP) should be performed when possible. ATP release by lumi-aggregometry is considered to be a reasonable screen for δ-SPD.

‘Third Line’ laboratory investigation

Depending on the suspected defect at this stage, specialized laboratories may be capable of performing additional sophisticated studies. These studies should be considered as confirmatory rather than screening. Examples include platelet electron microscopy to examine for the absence of dense granules in suspected δ-SPD. Routine application of this technique has suggested that ‘forme fruste’ variants of this disorder, in which there is a partial deficiency of dense granules, may be relatively prevalent in women with bleeding disorders. Other studies that may be of value in certain scenarios include platelet flow cytometry (e.g., in suspected Glanzmann’s or Bernard-Soulier syndromes), and specific studies of platelet release (e.g., serotonin release), receptor deficiency, or signal transduction.

Tailoring the work-up for bleeding disorders:

As a general rule, the more severe the bleeding disorder, the earlier in life the presentation. However, recent studies have begun to evaluate the clinical and laboratory features of mild bleeding disorders in children. These studies, like those in adults, conclude that platelet function defects (abnormal aggregation and/or ATP release) may account for a relatively large proportion of bleeding diatheses in children. In addition, it is clear that the cause of muco-cutaneous bleeding remains enigmatic in many children referred for evaluation of seemingly abnormal muco-cutaneous bleeding (14).

Racial differences may also be an important consideration in the work-up of bleeding disorders. For example, it has been demonstrated that among African-American women presenting with menorrhagia, von Willebrand’s disease is less common, whereas intrinsic platelet function defects are more common.

The approach outlined by this review is recommended primarily for outpatients referred for evaluation. Even more complex is the evaluation of hospitalized in-patients, who are generally referred because of post-surgical bleeding. As already mentioned, it may be difficult or even impossible to establish a diagnosis of vWD in a stressed individual in the post-operative period. Once again, a careful history will provide information on whether the patient has experienced spontaneous or excessive post-surgery/trauma bleeding in the past, or has a positive family history of such. Understanding whether bleeding was uni-focal or multi-focal may suggest a surgical or coagulopathic cause, respectively. Other common acquired causes of bleeding in hospitalized patients, such as vitamin K deficiency, dilu-
tional coagulopathy, or DIC should be considered. However, ultimately, a laboratory evaluation may be indicated; in order to simplify test interpretation, it is recommended that this be postponed until the patient has been discharged if at all possible.

**Management of the patient with a positive bleeding history and an unremarkable laboratory evaluation**

Even after an extensive tiered laboratory investigation of a subject strongly suspected of having a bleeding disorder, no specific abnormality may be discovered. This outcome may be the case in 40% or more of patients believed to have a bleeding disorder on the basis of the history. This dilemma can present a therapeutic challenge, since many of these individuals are evaluated prior to elective surgery. The literature contains very little information on the management or outcomes in these subjects. Thus, a careful assessment of the risk/benefit and cost/benefit ratios of empirical use of anti-fibrinolytic agents, DDAVP, and/or platelet transfusions, or even recombinant FVIIa, must be implemented. Reminders to the patient to avoid all platelet inhibitory agents prior to the procedure, and to the surgeon to take extra care in securing intra-operative surgical hemostasis are worthwhile.

**The future**

Studies that aim to better standardize the clinical history and existing laboratory studies in the diagnosis of bleeding disorders will be of particular value. It is clear however that assays of platelet function with greater sensitivity and specificity are required. To this end, proteomics approaches that evaluate the range of proteins expressed by normal and abnormal platelets are a realistic expectation in the next decade. Similarly, it is reasonable to expect that the pharmaceutical armamentarium of hemostatic agents, which is currently quite limited, will be expanded to cope with the shifting paradigm demanding better diagnosis of the causes of bleeding, and avoidance of a simplistic transfusion approach when bleeding does occur.