Investigating people with mucocutaneous bleeding suggestive of primary hemostatic defects: a low likelihood of a definitive diagnosis?

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People with mucocutaneous bleeding represent a major subtype of hematologic clinical presentations but simultaneously present a substantial diagnostic challenge. On the one hand, bleeding symptoms are frequent in the general population, but their clinical relevance may be difficult to assess. For example, nosebleeds (epistaxis) are common (especially in children), and menorrhagia is not an uncommon presentation in women.[1-4] So, are such findings really significant in a patient presenting for clinical review? Sometimes yes, sometimes no. Conversely, some people with an unequivocal diagnosis of a known disorder (e.g. congenital von Willebrand's disease; VWD) may be relatively asymptomatic.[2,5]

If a significant disorder is clinically suspected, several steps need to be taken, including a physical examination, a clinical review, laboratory testing, and ultimately appropriate clinical management.[1-8] The clinician also initially needs to determine whether the presentation represents a congenital or acquired disorder, and whether it appears to be predominantly of *primary* or *secondary* hemostasis. There are currently several possible options with regards to the clinical bleeding review.[1-6,9,10] For laboratory investigation, tests associated with the evaluation of VWD and platelet function disorders or defects (PFD) comprise the most common approach, and would define the best characterized of the primary hemostatic disorders. However, even these fairly common tests entail significant diagnostic difficulties (*see later*).

Moreover, many patients with mucocutaneous bleeding have no diagnostically identifiable disease, even after repeated testing. This is very well highlighted in the current issue of Haematologica in the paper by Quiroga and colleagues,[2] who present findings from a large prospective study of patients with congenital mucocutaneous bleeding to determine the relative frequency of bleeding disorders, the prevalence and characteristics of patients with bleeding of known and unknown cause, and the diagnostic efficacy of a comprehensive laboratory investigation. This is the first large prospective and systematic study of the characteristics, frequency and pathogenesis of bleeding in such people. Despite significant clinical evidence of a bleeding diathesis, and extensive laboratory testing, most investigated patients could not be characterized into any specific diagnostic category. This is the latest of several studies related to mucocutaneous bleeding from this group of workers.[11-13] In the current study,[2] 280 consecutive patients with mucocutaneous bleeding were assessed along with 299 matched controls. Fifty patients (17.9%) were found to have VWD (mostly type 1 [n=45] with a few having type 2 [n=5]). PFD and mild clotting factor deficiencies were found in 65 (23.2%) and 11 (3.9%) patients, respectively. Thirteen (11.5%) patients had combined defects. The remaining 167 (59.6%) patients had bleeding of unknown cause, with a prolonged bleeding time in 18.6% as their only abnormality. All of the reported disorders, including those that could not be diagnosed, were clinically undistinguishable. Moreover, no relationship was found between the severity of bleeding and any of the VWF/platelet function variables. The study concluded that the diagnostic efficacy of a first laboratory testing in patients with hereditary mucocutaneous bleeding was 40.4%. Thus, most patients (~60%) have a disease(s) of high prevalence but of unknown pathogenesis. This main finding was not dissimilar to that determined in a preliminary retrospective study from the same group.[13]

Assessing bleeding status: early considerations

If a clinical bleeding diathesis is considered likely, the determination of whether the disorder has characteristics of a primary or secondary hemostatic defect, and whether it has a congenital basis (i.e. is genetically determined) or is an acquired disorder should be among the first clinical considerations, as this will help to determine the laboratory testing approach and will also influence subsequent clinical management. Primary hemostatic disorders are those that influence the interaction of blood platelets with the vessel wall, which concludes with the formation of the platelet plug. This is influenced primarily by the level and function of both von Willebrand factor (VWF) and platelets, and is expressed pathologically by VWD and PFD, respectively. Secondary hemostatic defects are those that involve the eventual formation of fibrin by the clotting or coagulation cascade, influenced primarily by the level and function of clotting factors such as factor VIII and fibrinogen, and are expressed pathologically by various hemophilias and hypo/dysfibrinogenemias. Primary and secondary hemostatic defects sometimes have overlapping clinical presentations (e.g. type 3 VWD), but primary defects tend to express bleeding in high-shear-pressure blood systems (thus mucocutaneous bleeds are common) whereas secondary defects tend to express bleeding in low pressure areas or deep tissues (thus the bleeding presents commonly as hematomas or post-surgical hemorrhage). Sometimes, however, pathological condi-

tions lead to a combination of presentations due to combined defects/deficiencies (e.g. loss of VWF and factor VIII in type 3 VWD).

It is important to determine whether a disorder is congenital or acquired, since management of these will often differ. Thus, management of congenital disorders (e.g. VWD and hemophilia) will typically involve replacement therapy,[8] whereas management of acquired disorders (e.g. acquired hemophilia or VWD) will alternatively or additionally involve other approaches (e.g. intravenous IgG therapy for acquired VWD)[14-16] if, for example, an immune basis can be established. For PFD, an acquired defect may simply require modification of drug therapy (if medication-related), whereas a congenital defect may require a more substantive intervention such as replacement or desmopressin therapy.[17,18]

In general, the major clues as to whether a condition is acquired or congenital are the timing of the bleeding event(s) together with a review of family members. Thus, if events have only been recent and short-term, with no evidence of a familial bleeding tendency, the condition is probably an acquired event. In addition to standard laboratory testing to establish whether an acquired VWD or PFD is present, additional evaluation should include an investigation into the putative primary event that triggered the secondary bleeding tendency (medication-related effect, secondary to an autoimmune event or primary malignancy, etc). Alternatively, if events have been longer term, and there is evidence of a familial bleeding tendency (in parents, siblings or cousins), the condition is probably congenital. It might be worthwhile investigating the other affected family members along side the original propositus to look for similar laboratory phenotypes. Again, the question of whether bleeding is congenital or acquired is not always as straightforward as one hopes; for example, difficulties can be encountered: (i) in the assessment of children whose histories are relatively short-term and hemostatic challenges have been limited; (ii) when family members are lacking; (iii) when the patient themselves are vague or unhelpful.

Assessing bleeding severity: clinical reviews and questionnaires

Irrespective of the origin of the disorder, a clinical assessment of bleeding severity is critical. As noted, there are several possible options for undertaking a clinical bleeding review.[1-6,9,10] In the study by Quiroga *et al.*,[2] the patients were interviewed by the same investigator using a standardized questionnaire, modified to assess mainly mucous and skin bleeding. The interviewer recorded the current and past bleeding episodes independently of the age at consultation, and the most frequent and typical symptoms were scored from 0 to 4, according to the frequency, duration, recurrence, and need and type of therapy. Other symptoms, of lesser frequency, less typical of primary hemostatic disorders, or those present only after exposure to risk, were scored 0 (absent) or 1 (present). The recorded data were reprocessed to then provide a numerical assessment of bleeding severity in a Bleeding Score (BS). The BS was contrasted with the insight of the physician who estimated, interpreted and classified the bleeding severity at the end of the interview into a Clinical Classification (CC) with five categories: intense, moderate, intermediate, and trivial bleeding and non-bleeders. In essence, the BS quantified the bleeding according to the patient's (or parents') point of view and the CC reflected the judgment and perception of the severity of the disease by the physician. Both assessments also considered the bleeding history of first and second-degree relatives.

The study characterized individuals as having CC scores of 1 [n= 138], 2 [n=95], or 3 [n=47], and a good correlation was observed between CC and BS scores. Significantly, neither the CC nor the BS, as indices of bleeding severity, was significantly correlated with any of the multiple variables of plasma levels of VWF or platelet function, considering either the whole population of patients or each diagnostic group separately. Furthermore, no differences in the proportions of CC 1, 2 and 3 scores were found among all diagnostic groups. So, from a practical, clinical point of view, the elaboration, application and analysis of a time-consuming and complex BS was not better than an interview with a pre-established questionnaire followed by the classification of the bleeding severity by the physician (CC).

Thus, while there is no doubt that all reported approaches[1-6,9,10] can appropriately characterize individuals at risk of bleeding, the approaches differ substantially in terms of the effort required. Whether the more complex or time-consuming approaches work better than the simpler approach used by many experienced clinicians is currently not known; however, the study by Quiroga *et al.*[2] suggests that the latter may be just as effective. Nevertheless, further studies are required. The question of whether the complex approaches are better suited to clinical studies (i.e. useful in predicting the average findings of a group of people) than to day-to-day clinical use (i.e. a physician undertaking an evaluation and making decisions about an individual patient) also needs to be addressed. As a close colleague recently surmised, the latter "*requires the old-fashioned skill of taking a thorough and probing history from the patient and exercising clinical judgement*".[19]

Laboratory issues and testing

As previously noted, the laboratory investigation of primary hemostatic disorders or mucocutaneous bleeding most typically involves tests to evaluate the possible presence of VWD or PFD. However, even these fairly common tests comprise significant diagnostic difficulties. There are several possible diagnostic approaches for the investigation of VWD and their efficacy differs.[7,20] A panel of different tests is required to diagnose

VWD appropriately, and the use of inadequate panels will result in the misidentification or misdiagnosis of a significant proportion of individuals. Different tests and methodologies also have differing diagnostic efficacy.[7,20] Test panels should be comprehensive and use the best available technology. From this author's perspective, laboratories that do not perform a VWF:CB assay run a relatively high risk of misidentification/misdiagnosis of VWD.[7,20] Standardization issues are also significant in the evaluation of VWD.[7,20,21]

Substantial problems are also evident with platelet function studies, including limitations to existing tests as well as standardization issues.[22-24] Indeed, no consensus exists regarding the standardization and interpretation of *in vitro* platelet aggregation/secretion studies, and this will influence diagnostic rates.[21,24] It is reassuring that a wide panel of appropriate tests and methodologies, as currently available, were employed in the study by Quiroga *et al*.;[2] thus the finding that some 60% of clinically identified patients could not be ascribed a diagnosis remains significant. However, we should remain positive that patients may be able to be better characterized in the future, given the emergence of newer technologies.[24,25]

Genetics, prevalence and geographic locality

Genetic testing is not informative in most people with primary hemostatic disorders. For example, most patients with VWD have type 1 VWD and most of these have no distinctive genetic markers;[26] the diagnosis rests on decreased plasma VWF level and function. However, genetic and acquired factors result in a wide distribution of plasma VWF, and in general, VWF levels show only a weak correlation with bleeding.[2,27] A proportion of non-bleeder individuals will also have VWF levels below the established normal range, simulating a type 1-VWD. The prevalence of VWD, although variable, depending on the diagnostic method used, has been conservatively estimated at 100 cases per million.[28] Irrespective of the actual prevalence, VWD is classically considered to be the most common inherited bleeding disorder, and hence would be expected to comprise most cases of mucocutaneous bleeders. This is our own local experience (*see below*).

The prevalence of PFD is essentially unknown, although platelet secretion and signal transduction defects are the most frequently reported forms.[2] Although severe congenital disorders such as Bernard-Soulier syndrome (BSS) and Glanzmann's thrombasthenia (GT) are generally considered relatively rare (especially among Caucasian populations), this may not hold true in some geographic regions. Thus, certain localities have fairly high incidences of these defects, typically because of consanguity.[29,30]

The prevalence of PFD in the study by Quiroga *et al*.[2] was actually found to exceed that of VWD, but local geographic referral conditions were noted as a possible confounding influence on these study findings. Thus, they noted from their records spanning a period of 26 years, that they had diagnosed 17 patients with GT and two patients with BSS, but only one patient with type 3 VWD. In contrast, from our own experience and referral base over the past 20 years we have assessed samples from over ten individuals with type 3 VWD, but fewer than five with GT or BSS. Similarly, the relative frequency of type 2 VWD in Quiroga's population (about 10%) was significantly less than that in pure Caucasian populations. Our own general experience with VWD suggests that, overall, around 30% have of type 2, but this rate may reach as high as about 50% over some test periods.[31] Others have also reported values closer to 50% for type 2 VWD.[5,32] Again, this all depends on both the make up of the local population as well as the specialized testing referral base. In our case, together with those of others such as Federici[8] and Budde *el al*.,[32] the unusually high proportion of cases type 2 VWD probably relates more to the referral base than to the population base (i.e. more diagnostically difficult samples are sent to these laboratories, so the overall test base reflects an increase in the less common population phenotypes). In any case, these differences would not be expected to explain the main finding of Quiroga *et al*.,[2] and the lack of a definitive diagnosis in some 60% of investigated patients is still likely to be confirmed in other localities should this be investigated.

Utility of the PFA-100® to assess primary hemostatic disorders and mucocutaneous bleeding

The PFA-100® has only a limited utility within the context of the assessment of primary hemostatic disorders/mucocutaneous bleeding, with additional utility during the management phase in some cases.[11,31,33-36] Although the PFA-100® has a relative high sensitivity for the detection of severe VWD and PFD, it has a low sensitivity for mild disorders. Furthermore, the PFA-100® has no specificity for any disorder. In other words, a negative finding (normal PFA-100® result) may preclude a severe primary hemostatic disorders, but so would a good clinical review. A negative finding will not necessarily preclude a mild primary hemostatic disorders, and hence additional specific testing for VWD or PFD would still be warranted in patients with a significant clinical history and a negative PFA-100® result. A positive finding (abnormal PFA-100® result) may or may not suggest a significant primary hemostatic disorder, and hence additional specific testing for VWD or PFD might still be required to define the precise diagnosis. Thus, use of the PFA-100® may not assist the clinician in many cases under investigation.

A PFA-100® test may be of some use in the following circumstances: (i) proximity problems: the main test laboratory (which performs specific tests for VWD/PFD) is remote from the referring clinician; (ii) time constraint issues (e.g. imminent surgery, requiring

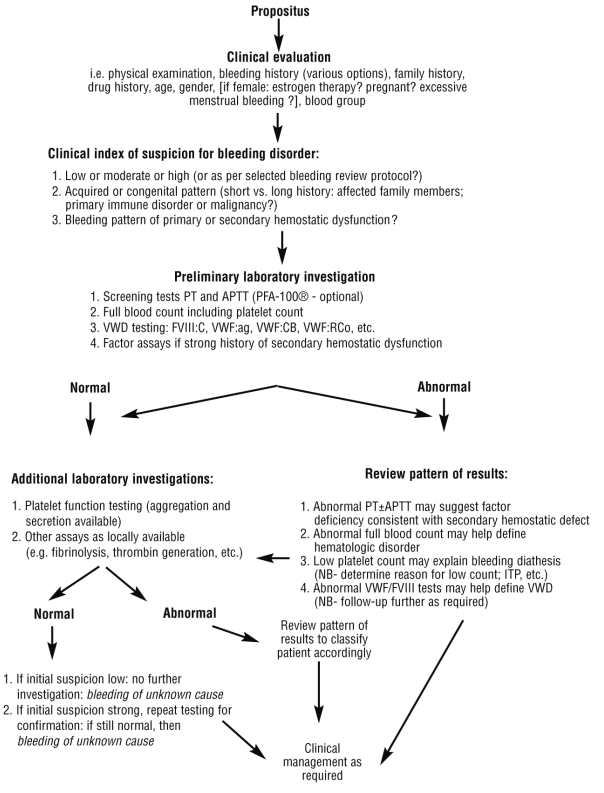

Figure 1. One possible approach to the investigation of a patient presenting for investigation of a bleeding diathesis. This figure should only be treated as a guide, and readers are encouraged to seek local expert opinion. Patients should be recognized as being individuals and assessed and managed on a case-by-case basis. The reader is also encouraged to seek additional guidance from the key references cited in the manuscript.

a decision to proceed or not in the case of limited clinical information, since the PFA-100® result is nearly immediate, but specific testing for VWD/PFD may take several days); (iii) vague or no clinical history available, or inexperienced clinician; (iv) monitoring of desmopressin therapy. In all other cases where good clinical histories are available, and sufficient time is available for a comprehensive laboratory work-up, testing with the PFA-100® can usually be omitted.

Final comments and the future

It is likely that the improved standardization of existing tests (e.g. for investigation of VWD and PFD) will improve the overall clinical diagnosis of primary hemostatic disorders.[20,21,24] Moreover, additional tests considered new, or emerging technologies may also improve such diagnoses (e.g. flow cytometry procedures, *overall hemostatic potential*, thrombin generation).[24,25] Despite this, a previous study by Quiroga *et al.*[12] did not find any significant findings in patients with hereditary mucocutaneous hemorrhages with respect to thrombin generation using platelet-poor plasma. Studies using platelet-rich plasma in such patients remain unpublished, but as thrombin generation is generally considered a predominant part of the pathway of secondary hemostasis, perhaps we should not expect too much within the context of currently 'unexplained mucocutaneous bleeding'?

There is, interestingly, a likely contribution of tissue fibrinolysis to mucocutaneous bleeding, as suggested by the effectiveness of antifibrinolytic drugs in many of these patients, without evidence of systemic hyperfibrinolysis. Indeed, in the study by Quiroga *et al.*,[2] shortened clot lysis time was detected in patients with PFD, and this deserves further study. Nevertheless, at this time, laboratory tests for tissue fibrinolysis have not really translated into effective diagnostic tools, either in most laboratories or for most investigations, and the diagnosis of congenital fibrinolytic defects remains generally elusive. Perhaps the as yet undiscovered fibrinolysis-based tests of the future will help to define a more significant subgroup of these patients.

Ultimately, it may never be possible to reach a clinical diagnosis for all patients with mucocutaneous bleeding. Apart from the (skin) bleeding time, current tests and emerging technologies focus only on the function of various blood components such as VWF and platelets. There is no good test for vascular integrity *per se*, nor are there any good tests to evaluate other possible blood components potentially involved in the bleeding diathesis characterizing these patients. The bleeding time test does measure vascular integrity to some extent (i.e. in combination with blood component functions), but it cannot be considered a good test. It is possible that endothelial or subendothelial integrity, or inherent dysfunction therein, may account for a proportion of primary hemostatic disorders that we cannot currently effectively diagnose. I wonder whether testing for this is perhaps the challenge of another generation of hemostasis scientists.

Figure 1 provides an algorithm of one possible approach to a patient presenting for investigation of a bleeding diathesis at the current time. This figure should only be used as a guide, and readers are encouraged to seek local expert opinion. For example, it should be recognized, that in our experience, VWD is more common than PFD. Since testing for VWD is also less onerous than testing for PFD in our institution, we always test for VWD first, and only investigate for PFD when results for VWD testing are not conclusive. In many other institutions, perhaps including that of Quiroga *et al.*,[2] testing for both VWD and PFD may occur simultaneously or even in a different order.

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