Clinical and laboratory diagnosis of von Willebrand disease

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Abstract

von Willebrand disease (VWD) is a hereditary bleeding disorder first described by Erik von Willebrand in 1926. The disease is characterized by frequent bruising, bleeding from minor wounds, nosebleeds, heavy menstrual bleeding, bleeding after tooth extraction, gastrointestinal bleeding, and joint bleeds. The underlying cause of VWD was identified 45 years later as a deficiency of von Willebrand factor (VWF), a high-molecular-weight protein that circulates with factor VIII in plasma. The clinical diagnosis of VWD involves the presence of bleeding symptoms, detection of at least one abnormality of VWF function in laboratory tests, and demonstration of familial inheritance. VWD presents a broad spectrum of clinical and laboratory abnormalities, making the diagnostic process complex. Integrating clinical and laboratory data into the diagnostic pathway using a Bayesian approach can help build evidence for a diagnosis. Over the years, several diagnostic assays have been developed to measure the quantitative and qualitative properties of VWF. Automated laboratory assays for measuring VWF have been implemented in recent decades, and assessment tools for clinical evaluation of bleeding severity have been developed. Laboratory diagnosis involves screening and first-level diagnostic tests to measure VWF antigen, VWF-platelet binding activity, and factor VIII coagulant activity. Second-level subtyping tests are required to characterize the phenotypic defects of the type 2 variants and classify the patients. Proper diagnosis and classification of VWD are essential in order to recognize patients who may benefit from treatment, determine the optimal treatment modality, and prevent overdiagnosis.

Introduction

When Erik von Willebrand described in 1926 a new hemorrhagic syndrome that he had recognized in a family from the town of Föglo, in the Åland Islands, he did not have standardized bleeding questionnaires or bleeding assessment tools at his disposal, nor any of the sophisticated laboratory tests that we can nowadays apply to make the diagnosis of von Willebrand disease (VWD).[1,2]

The clinical symptoms encountered in the family described by von Willebrand were frequent bruising, bleeding from minor wounds, nosebleeds, heavy menstrual bleeding, bleeding after tooth extraction, gastrointestinal bleeding, and joint bleeds. The bleeds could be severe and, in several cases, fatal. He recognized the familial nature of the disease but was uncertain about the cause and named it “hereditary pseudo-hemophilia.”[1] Forty-five years later, the disease was associated with a deficiency of a high-molecular-weight protein, von Willebrand factor (VWF), independently synthesized and secreted from factor VIII (FVIII) but tightly bound to it in plasma.[3]

Since the elucidation of the main pathophysiology of the disease, several diagnostic assays have been developed over the years to measure quantitative and qualitative properties of VWF. New automated laboratory assays for measuring VWF have been developed and implemented in recent decades.[4] By the early 2000s, the development of assessment tools for clinical evaluation of bleeding severity had been addressed.[5,6] Proper diagnosis and classification of VWD are essential to recognize patients who may benefit from treatment, to determine the optimal treatment modality, and to prevent overdiagnosis.[7-10] This review discusses the current state-of-the-art clinical and laboratory diagnosis of VWD.

Clinical diagnosis of von Willebrand disease

The presence of bleeding symptoms, detection of at least one abnormality of VWF function in laboratory tests, and demonstration of familial inheritance have been considered the diagnostic triad clinically defining VWD.[11] Unfortunately, VWD presents a broad spectrum of clinical and laboratory abnormalities, and the diagnostic process may range from extremely easy to extraordinarily complex. The latter case occurs when a patient has only a few bleeding symptoms and a mild reduction of VWF levels (i.e., between 30 and 50 IU/dL), a situation known as "possible VWD" or "low VWF."[12] Furthermore, in VWD patients, the association between bleeding symptoms and reduced VWF levels usually becomes evident only for VWF levels well below 30 IU/dL,[13-16] thus making possible the dissociation between the laboratory and clinical phenotypes (a phenomenon also known as "variable penetrance"). Finally, the laboratory phenotype tends to improve or normalize in patients with a low VWF as they age, explaining why some adult patients may have a diagnosis of VWD made during their youth.[17]

Integrating clinical and laboratory data into the diagnostic pathway

Rather than relying on fixed criteria, a Bayesian approach builds evidence for a diagnosis from already available data. This usually means the same evidence may have different diagnostic implications, depending on the *a priori* setting.[18] For instance, a history of even mild bleeding symptoms may be strongly suggestive of VWD in a young child or siblings of a VWD patient. At the same time, it may be irrelevant in an elderly subject from an unselected population. The pre-test setting confers different *a priori* probabilities of having VWD, the highest *a priori* probability being that of VWD siblings who have a 50% probability of having VWD themselves (or a 1:1 odds of being affected, that is, they are equiprobably affected by VWD or healthy). In contrast, subjects from an unselected population have a small chance of being affected by VWD: assuming a VWD prevalence of 0.1%, their corresponding odds of being healthy are 99,999 to 1.[19]

In addition to the above two settings, the most frequent and challenging clinical situation is when the patient reports at least some bleeding symptoms (Table 1). Although the prevalence of VWD in these patients is not known precisely, it could be estimated to be in the range of 10-20%,[12,19,20] thus making the diagnosis of VWD very relevant in this subset of patients.

Clinical assessment of bleeding symptoms

A thorough assessment of bleeding symptoms requires the appraisal of both the frequency and severity of the symptoms in the individual patient. Since the bleeding phenotype in VWD patients is most often mild to intermediate, the physician should help the patient in this retrospective exercise, keeping in mind that the recall bias may lean toward under-appreciation for affected family members (who may dismiss some symptoms because they are considered as a normal family phenotype) or toward over-appreciation for anxious individuals. Few studies are available reporting the incidence of bleeding symptoms in normal individuals, and it should be remembered that the relative importance of bleeding symptoms may vary with age.[21,22]

Cutaneous bleeding (e.g., prolonged bleeding after minor cuts, presence of ecchymoses or subcutaneous hematomas), post-surgical bleeding, menorrhagia, epistaxis, and bleeding after teeth extractions are the bleeding symptoms more frequently reported by VWD patients as compared to age- and sex-matched controls and should be thoroughly investigated in patients who are candidates for a diagnosis of VWD.[13] Additionally, valuable criteria to discriminate when bleeding symptoms are relevant were discussed by a joint committee of the International Society of Haemostasis and Thrombosis (ISTH),[23] as reported in Table 2.

When at least three different bleeding symptoms are present, the patient's clinical history is considered suggestive of the presence of a bleeding disorder (especially VWD).[24] Alternatively, the Bleeding Score is a semi-quantitative bleeding assessment tool (BAT) that can be useful for condensing the patient's bleeding phenotype into a single measure.[5] Initially developed for research purposes,[13,25] it was subsequently standardized by the ISTH to assess not only the most severe bleeding symptoms but their frequency as well (ISTH-BAT[23]). The use of a standardized BAT may assist in the diagnosis of bleeding disorders,[20] and may predict future bleeding in VWD patients.[26] Although the positive predictive value of the Bleeding Score has never

Table 1. Diagnostic scenarios for von Willebrand disease.

Scenario	Pre-test probability of VWD	Examples
Unselected patients	Low (< 1:1,000)	Primary care Presurgical screening
Patients referred because of the presence of bleeding symptoms	Intermediate (10-20%)	Secondary referral clinics (e.g., Hemophilia Center) Patients with post-surgical or post-partum bleeding
Family studies	High (≈50%)	Offspring of an affected individual

VWD: von Willebrand disease.

Table 2. Prevalence of selected bleeding symptoms in patients with von Willebrand disease and normal individuals.

Symptom	Prevalence in VWD patients, %	Prevalence in normal individuals, %	ISTH criteria that define as clinically relevant[23]
Cutaneous bleeding	30-90	2-34	Bruises: five or more (>1 cm) in exposed areas; petechiae: when adequately described by the patient or relatives; hematomas: occurring without trauma.
Post-surgical bleeding	10-60	0-6	Any bleeding judged by the surgeon to be abnormally prolonged that causes a delay in discharge or requires treatment.
Menorrhagia[#]	2-95	25-53	Any bleeding that interferes with daily activities such as work or social activities during most menstrual periods. Criteria for significant bleeding may include changing pads <2 hours, lasting >7 days, or flooding with clots. Alternatively, a pictorial blood loss assessment chart (PBAC) score >100.[71]
Epistaxis	12-80	2-57	Any nosebleed, mainly occurring after puberty, that causes interference or distress with daily or social activities.
Bleeding after teeth extractions	40-60	3-12	Any bleeding that occurs after leaving the dentist's office requiring a new, unscheduled visit or prolonged bleeding at the dentist's office that causes a delay in the procedure or discharge.

[#]in female patients. Data abstracted from.[72-79] VWD: von Willebrand disease; ISTH: International Society on Thrombosis and Haemostasis.

been proven to be superior to the simple evaluation of the number of bleeding symptoms reported by the patient,[24] the final likelihood of VWD increases with the patient's Bleeding Score.[27] The cut-off for a positive or abnormal Bleeding Score is ≥4 in adult males, ≥6 in adult females, and ≥2 in children.[28,29] An obvious limitation of the ISTH-BAT is its low sensitivity in a pediatric setting.[30]

Laboratory diagnosis of von Willebrand disease

When there is suspicion of VWD, based on either a personal bleeding history or a family history, further laboratory testing is indicated. The approach to the laboratory diagnosis of VWD can be divided into two parts: first, establishing the presence or absence of VWD, and second, characterizing the type of defect to classify the disease into any subtypes.

Screening tests

Several hemostatic screening tests will be performed when a patient is suspected of having a bleeding disorder. Usually, these tests include screening of secondary hemostasis through the activated partial thromboplastin time (aPTT) and prothrombin time, and screening of primary hemostasis with a platelet count and potentially a platelet function analyzer test (PFA-100® or the newer PFA-200®), although the latter may not be available in all centers.[31] For many years, the so-called bleeding time was used as an *in vivo* screening tool for primary hemostasis. However, due to interobserver variability, low sensitivity and specificity, and the burden of this invasive test for the patient, the bleeding time has been abandoned and is now considered obsolete. These screening tests will give a general impression of the hemostasis of a patient and are mainly intended to direct the subsequent analysis but are not specific for VWD. In the case of VWD, a prolonged aPTT may be identified when the factor VIII (FVIII) activity level is reduced.[32] Therefore, a prolonged aPTT may prompt more specific tests, but a normal aPTT does not rule out VWD. Low platelet counts can be found in association with VWD type 2B.[33] A prolonged closure time in the PFA-100® could suggest VWD or a platelet defect; however, a normal PFA-100® does not exclude milder cases of VWD, and subsequent specific analysis will be required when the suspicion of VWD is real.[34]

First-level diagnostic tests

To either confirm or reject the diagnosis of VWD, the laboratory test panel consists of measuring three analytes in plasma: the amount of VWF antigen (VWF:Ag), the VWF-platelet binding activity, and the FVIII coagulant activity (FVIII:C). Some laboratories also include VWF collagen binding activity (VWF:CB); however, most guidelines, including the recent international guideline, suggest the three-test panel.[12]

von Willebrand factor antigen

Several assays are being used to measure VWF:Ag: an enzyme-linked immunosorbent assay (ELISA), an ELISA-based chemiluminescent immunoassay (CLIA), and an automated latex immunoassays (LIA). The ELISA and LIA are comparable, however, with a lower detection limit for the ELISA but a better coefficient of variation for the LIA.[35] Currently, most laboratories use the automated LIA.[36]

von Willebrand factor platelet-binding activity

One of the main properties of VWF is its binding to platelets. However, in a steady state, VWF circulates in the blood in its inactive globular form and cannot bind to platelets unless VWF is activated. Upon vascular damage, VWF will bind to the exposed subendothelial collagen matrix, uncoil, and be

activated. When activated, the A1 domain of VWF becomes exposed and can interact with the glycoprotein Ib (GPIb) receptor on the platelet surface. Weis *et al*. described the first assay to quantify this functional platelet binding activity of VWF using ristocetin to activate VWF and measuring the ristocetin-induced aggregation of washed platelets to quantify the VWF activity.[37] This assay, later known as VWF ristocetin cofactor activity (VWF:RCo), has been the gold standard until recently. In the last two decades, several new automated assays to reflect platelet-binding activity with higher precision and lower detection limits have been introduced in diagnostic laboratories as a substitute for the laborious VWF:RCo assay. These newer assays measure the binding of VWF to a recombinant GPIb fragment instead of binding to GPIb on platelets. One variation of this platelet-binding activity assay, the VWF:GPIbR assay, measures the binding of VWF after activation by ristocetin to recombinant wild-type GPIb and the other, the VWF:GPIbM assay, measures the spontaneous binding of VWF, without activation by ristocetin, to a gain-of-function mutant GPIb fragment.[38] It has been described that the activation of VWF by ristocetin may underestimate the actual VWF-platelet binding activity in carriers of a VWF polymorphism p.D1472H because the binding of ristocetin to the VWF A1-domain is reduced in those individuals.[39] This may affect the results of the VWF:RCo assay, but not the VWF:GPIbM;[40] however, the impact on the automated VWF:GPIbR is not yet clear. Finally, there is also a LIA based on a monoclonal antibody that recognizes the epitope in VWF where VWF binds to GPIb. Although this assay correlates with the results of the platelet-binding activity assays, it is not an assay that measures function. The VWF subcommittee of the scientific and standardization committee (SSC) of the ISTH suggested VWF:Ab as the nomenclature for this assay as it measures antibody binding and not an activity (Table 3).[38]

In a recent systematic review, the platelet-binding activity assays, VWF:GPIbR, VWF:GPIbM, and VWF:RCo, were compared.[41] The diagnostic test accuracy was comparable between all assays; however, the newer assays have a lower limit of detection than the VWF:RCo. Based on a lower coefficient of variation and a higher reproducibility of the VWF:GPIbR and VWF:GPIbM assays, the ASH ISTH NHF WFH guideline committee considered that the newer assays have a net benefit and suggested the use of the VWF:GPIbM and VWF:GPIbR assays over the VWF:RCo assay.[12]

Factor VIII activity

VWF is the natural carrier protein for circulating FVIII. Therefore, measuring FVIII activity is also part of the first-level test panel as FVIII may be variably reduced in all types of VWD, either due to a decrease in VWF level or a reduction in VWF binding affinity for FVIII. FVIII activity is usually measured as the coagulant activity (FVIII:C) by an automated one-stage clotting assay. However, two-stage clotting assays and chromogenic assays are also available.

Interpretation of first-level tests

When all three first-level tests are within the normal range, VWD is excluded. Usually, levels ≥50 IU/dL are used as a cut-off.[12] However, VWF and FVIII levels may vary over time and can increase because of an acute phase response, inflammation, or pregnancy; therefore, repeated measurements may be indicated depending on the clinical situation and the level of clinical suspicion of VWD. In some studies, VWF shows variability with the menstrual cycle, with lower levels during menstruation and the early follicular phase.[42] Further analysis and subtyping are suggested when one of the first-level tests is <50 IU/dL (see Table 4 for subtyping criteria). In some rare cases of VWD all first-level tests may be within the normal range, but with VWF platelet-binding activity-to-VWF:Ag ratio <0.7. In those cases, a rerun of first-level tests and further subtyping may be indicated.

When VWF:Ag levels are (virtually) undetectable, this is conclusive for diagnosing type 3 VWD. Detectable but reduced VWF:Ag levels that align with the reduction of the VWF-platelet binding activity suggest the presence of a mild quantitative deficiency and are classified as type 1 VWD. When one of the first-level tests is <50 IU/dL and there is a discrepant, more pronounced decrease of the VWF platelet-binding activity compared to VWF:Ag, this indicates a functional defect of VWF and falls under type 2 VWD. When the FVIII:C is disproportionately low compared to VWF:Ag this may indicate a functional defect of VWF in binding FVIII (type 2N VWD), but may also be indicative of mild hemophilia A. Additional second-level tests will be required for further subtyping of type 2 VWD.

As VWF and FVIII are continuous biological variables, it is not easy to define strict diagnostic cut-off levels. Already in 2003, Evan Sadler started a discussion that it is impossible to force patients into binary “diseased” or “healthy” categories.[43] Later, the term ‘low VWF’ became used to refer to a risk factor for bleeding rather than a disease.[44]

Table 3. Nomenclature approved by the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis.

Assay	Activator	Description of assay
VWF:RCo	Ristocetin	Assays that use platelets and ristocetin to measure binding of VWF to GPIb on platelets
VWF:GPIbR	Ristocetin	Assays based on the ristocetin-induced binding of VWF to a recombinant wild-type GPIb fragment
VWF:GPIbM	No activator	Assays based on the spontaneous binding of VWF to a gain-of-function mutant GPIb fragment
VWF:Ab	No activator	Assays based on the binding of a monoclonal antibody to the GPIb binding site in VWF (A1 domain epitope)

Adapted from Bodó *et al.*[38] VWF:RCo: von Willebrand factor ristocetin cofactor; GP: glycoprotein; Ab: antibody.

Table 4. Laboratory criteria for subtyping von Willebrand disease.

Type of VWD	Laboratory criteria
No VWD	VWF:Ag, VWF activity*, FVIII:C all ≥50 IU/dL
Type 1	FVIII:C/VWF:Ag and VWF activity/VWF:Ag ratio both ≥0.7 VWF:Ag or VWF activity <30 IU/dL regardless of bleeding symptoms
Type 1**	FVIII:C/VWF:Ag and VWF activity/VWF:Ag ratio both ≥0.7 VWF:Ag or VWF activity 30-50 IU/dL in the presence of bleeding symptoms (in the case of a positive family history, type 1 can also be considered)
Type 2A	VWF activity/VWF:Ag ratio <0.7 Abnormal VWF multimers, normal or reduced RIPA
Type 2B	VWF activity/VWF:Ag ratio <0.7 Abnormal VWF multimers, enhanced RIPA
Type 2M	VWF activity/VWF:Ag ratio <0.7 Normal VWF multimers
Type 2N	FVIII:C/VWF:Ag ratio <0.7 Abnormal VWF-FVIII binding
Type 3	VWF:Ag and VWF activity undetectable

*VWF activity denotes any assay measuring VWF platelet-binding activity. **This category has also been indicated in previous literature as 'Low VWF'. VWD: von Willebrand disease; VWF: von Willebrand factor; Ag: antigen; FVIII:C: factor VIII coagulant activity; RIPA: ristocetin-induced platelet agglutination assay.

When VWF:Ag and/or VWF platelet-binding activity is <30 IU/dL, there is little debate that this causes a bleeding disorder[14,45] and the patient should be diagnosed as having VWD, which is also in line with recent VWD guidelines.[46] However, when VWF:Ag and VWF platelet-binding activity are equally decreased and in the 30-50 IU/dL range, there is more debate regarding the diagnosis. Those patients could be diagnosed as having type 1 or 'low VWF'. In the current guideline, it was recommended to diagnose type 1 VWD regardless of bleeding symptoms when VWF:Ag and/or VWF platelet-binding activity is <30 IU/dL and to diagnose those with levels between 30-50 IU/dL as having type 1 VWD only in the presence of abnormal bleeding.[12] The guideline panel considered that labeling the latter category of patients as having VWD type 1 would benefit them because they would have better access to care in specific health systems.

When the first-level test of activity, either VWF platelet-binding activity or FVIII activity, is disproportionately low compared to VWF:Ag patients will be classified as having VWD type 2. However, which VWF platelet-binding activity-to-VWF:Ag ratio or FVIII activity-to-VWF:Ag ratio should be used as a cut-off is a matter of debate. Different cut-offs have been described in the literature. In a recent meta-analysis the sensitivity and specificity of different cut-offs for the VWF platelet-binding-to-VWF:Ag ratio were analyzed.[47] Based on the data from that meta-analysis, the recent guideline suggested using a VWF platelet-binding activity-to-VWF:Ag ratio <0.7 to confirm type 2 VWD (2A, 2B, or 2M).[12] However, the certainty of this advice is low. A lower cut-off will generally be more specific to identify type 2 but less sensitive. However, when type 2 is suspected, additional second-level subtyping tests will be performed anyway, so a more sensitive cut-off is preferred.

Second-level subtyping tests

Second-level tests are required to further characterize the phenotypic defects of the type 2 variants and classify patients as having type 2A, 2B, 2M, or 2N (Table 4). The tests measure structure and specific binding activities of VWF.

von Willebrand factor multimer analysis

A decreased VWF platelet-binding activity-to-VWF:Ag ratio <0.7 is indicative of types 2A, 2B, and 2M VWD. To distinguish types 2A and 2B from 2M, VWF multimerization needs to be evaluated. Multimerization patterns and defects can be visualized using VWF multimer analysis. Non-reduced plasma samples are run by electrophoresis on agarose-sodium dodecylsulfate gels of variable resolution (often 1.4-2.0% medium-resolution and 0.7-1.2% low-resolution gels). These assays are usually in-house methods with visualization by western blot, but newer assays have also been studied.[48,49] The multimers are evaluated mainly for the distribution of low- to high-molecular-weight multimers, and aberrant patterns of individual multimeric bands will also be visualized. In types 2A and 2B, the high-molecular-weight multimers are lacking, which causes the reduced platelet-binding activity. In contrast, in type 2M, the whole range of multimers is present, and the reduced platelet-binding activity results from a reduced binding capacity of the VWF A1 domain for platelet GPIb and not from the lack of large multimers.

von Willebrand factor collagen binding

Large VWF strings bind to collagen, and an ELISA or automated CLIA can test this VWF capacity. The binding of VWF to collagen (collagens type I and III) is very sensitive to the lack of high-molecular-weight multimer and, therefore, the VWF:CB-to-VWF:Ag ratio will also be decreased <0.7 in types 2A and 2B, whereas the presence of the

high-molecular-weight multimers in type 2M will result in a normal VWF:CB-to-VWF:Ag ratio.[12] As such, the VWF:CB can distinguish these types similarly as VWF multimer analysis, and the VWF:CB may be a substitute for multimer analysis. The subtype 2M also includes some rare cases in which the collagen binding is reduced due to an intrinsic defect in the collagen-binding domain of VWF, and these cases will have normal VWF multimers as well as normal VWF platelet-binding activity-to-VWF:Ag ratio.[50] Although not always included in the diagnostic work-up, the VWF:CB could have added value.

Ristocetin-induced platelet agglutination

Both types 2A and 2B VWD have abnormal multimers. However, type 2B is characterized by an increased affinity for GPIb, which can be detected using the ristocetin-induced platelet agglutination (RIPA) test.[51] VWF will not bind to platelet GPIb spontaneously unless VWF is activated. Ristocetin can induce *in vitro* activation of VWF after which VWF can bind to platelet GPIb and cause agglutination. At high concentrations of ristocetin, VWF will be activated and bind to GPIb; however, in type 2B VWD, with its increased binding affinity for GPIb, much lower concentrations of ristocetin will already induce platelet agglutination. In this way, the RIPA assay distinguishes between types 2A and 2B, with lower concentrations of ristocetin leading to agglutination in type 2B. There are also gain-of-function variants in the platelet GPIb receptor, known as platelet-type VWD, which also result in agglutination at low ristocetin concentrations in the RIPA assay and may be misdiagnosed as type 2B.[52]

von Willebrand factor-factor VIII binding

When FVIII activity is disproportionately low compared to VWF:Ag (reduced FVIII activity-to-VWF:Ag ratio <0.7), this may indicate type 2N VWD or mild hemophilia A. To make this diagnostic distinction, a VWF-FVIII binding (VWF:FVIIIB) assay can be performed. Often, these are ELISA developed in-house, but a commercial assay has been reported.[53,54] In type 2N VWD, the VWF:FVIIIB is clearly reduced, whereas it is normal in hemophilia A and other types of VWD. Some type 2A variants, mainly with mutations in the D3 domain, may have a mild reduction in VWF:FVIIIB, however, it is not a distinctive feature in those variants and the FVIII activity-to-VWF:Ag ratio will generally be within the normal range.

von Willebrand factor propeptide

The amount of VWF propeptide (VWFpp) in plasma can be measured by ELISA methods. VWFpp does not reflect an activity but the concentration of the circulating propeptide. VWF and VWFpp are secreted into the circulation in equimolar amounts, but their half-lives differ greatly; 8-12 hours for VWF and 2 hours for VWFpp.[55-57] The ratio between VWFpp and VWF:Ag can be used to assess enhanced clearance of VWF.[58] The VWFpp is not part of the formal classification; however, an increased VWFpp-to-VWF:Ag ratio may add to the diagnosis of patients with VWF variants with enhanced clearance and short half-life of VWF. These patients are sometimes designated as having type 1C VWD. The international ASH ISTH NHF WFH guideline on diagnosis of VWD suggests using a desmopressin (DDAVP) trial with 1- and 4-hour VWF:Ag measurements rather than the VWFpp-to-VWF:Ag ratio to confirm increased clearance.[12] Enhanced clearance is a characteristic of many VWF variants (also in type 2A and type 2B); however, in those cases, the enhanced clearance is not the main feature of the variant.[59]

Perspectives

In 1994, Evan Sadler published a revised classification of VWD that reduced the number of subtypes reported at that time from over 25 to only six (types 1, 2A, 2B, 2M, 2N, and 3).[60] That classification is still valid today, as it broadly separates patients with different needs regarding therapeutic management and counseling, which should be the primary goal of a correct VWD diagnosis.

Even though the laboratory criteria, as summarized in Table 4, may seem clear, we recognize that this classification scheme is not always straightforward, with some cases demonstrating phenotypic overlap between different type 2 subtypes.[61,62] The diagnostic challenge lies in determining significant bleeding risk and applying diagnostic cut-off levels for intrinsically continuous laboratory variables with care. Clinical experience is required to apply these criteria sensibly and not use them as absolute values.

From the diagnostic point of view, evaluating the *a priori* probability of VWD in the patient is relevant. As mentioned before, patients with a family history of VWD or having at least three different, non-trivial bleeding symptoms (or an abnormal Bleeding Score) should always undergo laboratory screening for VWD. This does not imply that patients who do not fall into the above clinical categories should never be tested, as about half of the obligatory carriers of type 1 VWD do not report any clinical symptoms yet may be at bleeding risk.[24] For instance, a recent meta-analysis reported that the prevalence of VWD in women with menorrhagia is 5 to 24%, with bleeding being the only symptom in about half of them.[63] Therefore, testing for VWD may also be considered in young patients with few bleeding symptoms but no apparent associated pathology (e.g., regular pelvic examination or rhinoscopy findings), as the *a priori* probability of a bleeding disorder in such a setting is increased.[22] Contrariwise, testing in a patient without any clinical clue suggesting VWD should be strongly contraindicated, as the expected rate of false positives is at least 7.3% (that is, the probability of finding either VWF platelet-binding activity, VWF:Ag or FVIII:C below the normal range). Despite the importance of introducing standardized BAT, especially for research purposes or to improve reproducibility between

assessors, there remains a significant knowledge gap in finely estimating the *a priori*, or pre-test, probability of VWD in secondary care. It should be explored whether the availability of large, annotated databases of VWD patients may improve current BAT in the coming years.[64-66]

Laboratory testing in VWD is as necessary to drive therapeutic decisions as it is to make the diagnosis. Distinguishing different (sub)types of VWD is relevant for estimating bleeding risk, choosing proper treatment, and counseling patients regarding the inheritance of the trait. Evaluating the VWD subtype and the pattern of VWF increase after DDAVP infusion is relevant for patients' management. Most patients with type 1 VWD can be treated with DDAVP, but some have an enhanced VWF clearance (indicated as type 1C), making concentrates preferable when sustained VWF/FVIII:C levels are necessary.[67] In type 2 VWD patients, DDAVP releases dysfunctional VWF into the circulation, which may not result in a significant increase in VWF platelet-binding activity, or it may even be contraindicated, as it may induce severe thrombocytopenia in type 2B VWD.[68] However, in some type 2A and 2M cases, the increase of VWF platelet-binding activity after DDAVP may be sufficient, especially for milder bleeding events or minor interventions.[68] In type 2N, the increase of VWF may be adequate, but the rise in FVIII may be very short-lived due to the lack of binding of FVIII to VWF.[69] In both types 2B and 2N, treatment with VWF concentrate is more appropriate. Identifying type 2N is also essential for distinguishing it from mild hemophilia A, which will require different treatment and genetic counseling.

From a clinical perspective, one could argue that a more straightforward classification based only on the pattern of DDAVP response may be sufficient. For instance, type 2A patients have a significantly higher risk of gastrointestinal bleeding than type 2M patients[70] but, since they share a similar management, differentiating between these two subtypes may be futile. Ongoing research on VWF and VWD will evidently yield new information, but we should be very restrictive about changing (and complicating) the current classification unless the new clinical and/or management data really change the paradigm.

Finally, it should be kept in mind that in patients previously referred to as having "low VWF"" (i.e., those having VWF levels between 30 to 50 IU/dL at diagnosis), an increase in VWF levels is to be expected with age. In these patients, careful monitoring of VWF/FVIII:C activities is required, especially before planned surgery, to avoid over-treatment and a possible risk of thrombosis. However, currently, there is no consensus on how to manage those VWD patients with normalized VWF levels, and this remains an area for future research.

Notwithstanding our progress in understanding VWD pathophysiology and diagnosis, we should acknowledge that the current tools for precisely estimating the bleeding risk and tailoring optimal therapies are inadequate. Significant aspects contributing to the physiology of VWF in hemostasis, such as blood flow, shear stress, and local release of VWF from activated endothelial cells and platelets at the site of vascular injury, are not reflected by the current diagnostic armamentarium and classification. Better functional assays, considering those aspects, are urgently needed and deserve validation for clinical use in the coming years.

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Contrinutions

Both authors contributed equally to this paper.

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