

## Diagnostic algorithm for von Willebrand Disease (vWD) in a Mexican population

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### Abstract

The diagnosis of von Willebrand disease (vWD) is complex and requires several screening and confirmation tests, such as the analysis of vWF multimers, which is considered the gold standard for vWD subtyping; however, it only discriminates 2A subtype while the 2B, 2M, and 2N subtypes require additional tests and even genetic testing for final confirmation. It is important to consider the patients' hemotype for the vWD diagnosis, particularly in Mexico where hemotype "O" predominates and may entail a 20-25% decreased level of plasma vWF and increased bleeding tendency. (Gac Med Mex. 2015;151:372-5)

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### VWD overview

Von Willebrand Disease (vWD) is the most common hereditary hemorrhagic disorder, with a worldwide prevalence of 1%, and it is the result of quantitative or qualitative defects of the von Willebrand factor (vWF). In spite of its high frequency, many vWD cases remain undiagnosed or are erroneously classified as mild or moderate hemophilia, due to the complexity and high cost of diagnostic testing, as well as to characteristics of the disease itself. For example, a 20-25% decrease in plasma vWF, which normally occurs in individuals with type O blood, could mask a mild quantitative vWD. Therefore, blood type is a trait that has to be considered, especially in Mexico, where blood type O has a frequency of 82% in open Mestizo population<sup>1</sup>.

This brief review is intended to integrate international recommendations and the experience from recent national works to propose a vWD diagnostic algorithm focused on our population's characteristics.

### Diagnosis by screening and confirmatory tests

vWD is initially suspected with a previous story of hemorrhages (characteristically mucocutaneous) in the patient and/or the family, together with coagulation tests (partial thromboplastin time, prothrombin time, Ivy bleeding time [BT], platelets). Although these are screening tests, they don't diagnose vWD; they are required because they can support its suspicion and/or rule out other coagulation disorders<sup>2</sup>. According to the

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International Society of Thrombosis and Hemostasis (ISTH), at least two hemorrhagic symptoms are required to be present in the absence of blood transfusion, or one symptom that required blood transfusion, or one recurrent symptom on at least three different occasions for the bleeding tendency to be considered significant and suspicious of vWD<sup>3</sup>.

The next step consists in having confirmatory tests made, including FVIII coagulant activity (FVIII:C) measurement, vWF activity evaluation (usually with the ristocetin-induced platelet aggregation assay, or vWF:RCo) and assessment of the vWF antigen (vWF:Ag). The relationship of the values of these last two parameters allows for the diagnosis to be established and to classify the vWD as quantitative (types 1 and 3) or qualitative deficiency (type 2)<sup>2</sup>. Given the broad variation of vWF plasma concentration owing to different physiological and environmental factors, to avoid biases in the vWF:RCo/vWF:Ag ratio, it is important performing these measurements in samples obtained in a single take. It should be underscored that, in spite of its availability in social security institutions, the poor resolution of the vWF:RCo assay requires extreme precautions to be taken for its interpretation. Thus, repeating this assay is recommended in case of diagnostic discrepancy, i.e., when the vWF:RCo/vWF:Ag doesn't agree with the verification performed using the multimer pattern<sup>4</sup>.

### **Analysis of vWF multimers**

The analysis of vWF multimers is considered the gold standard for vWD sub-typing, since it allows for vWD quantitative variants to be defined at low resolution and for 2A, 2B, 2M and 2N qualitative variables, to be defined at high resolution by separating in detail low molecular weight multimers<sup>2</sup>. Although this assay is intended to identify some variants of type 2 vWD with multimer structural alterations, it allows for some type 1 cases (mild to serious) to be confirmed with due consideration to the screening and confirmatory tests results. We recommend the use of pools of the type of blood of each patient in order to have a normal control according to the specific blood type, since the pattern of decreased intensity characteristic of normal subjects with blood type O can be mistaken with type 1 vWD in individuals with blood types other than O<sup>4</sup>. The 2A subtype, characterized by a multimer pattern with no intermediate and high molecular weight aggregates, is the only variant that can be directly and clearly discriminated with the multimer assay.

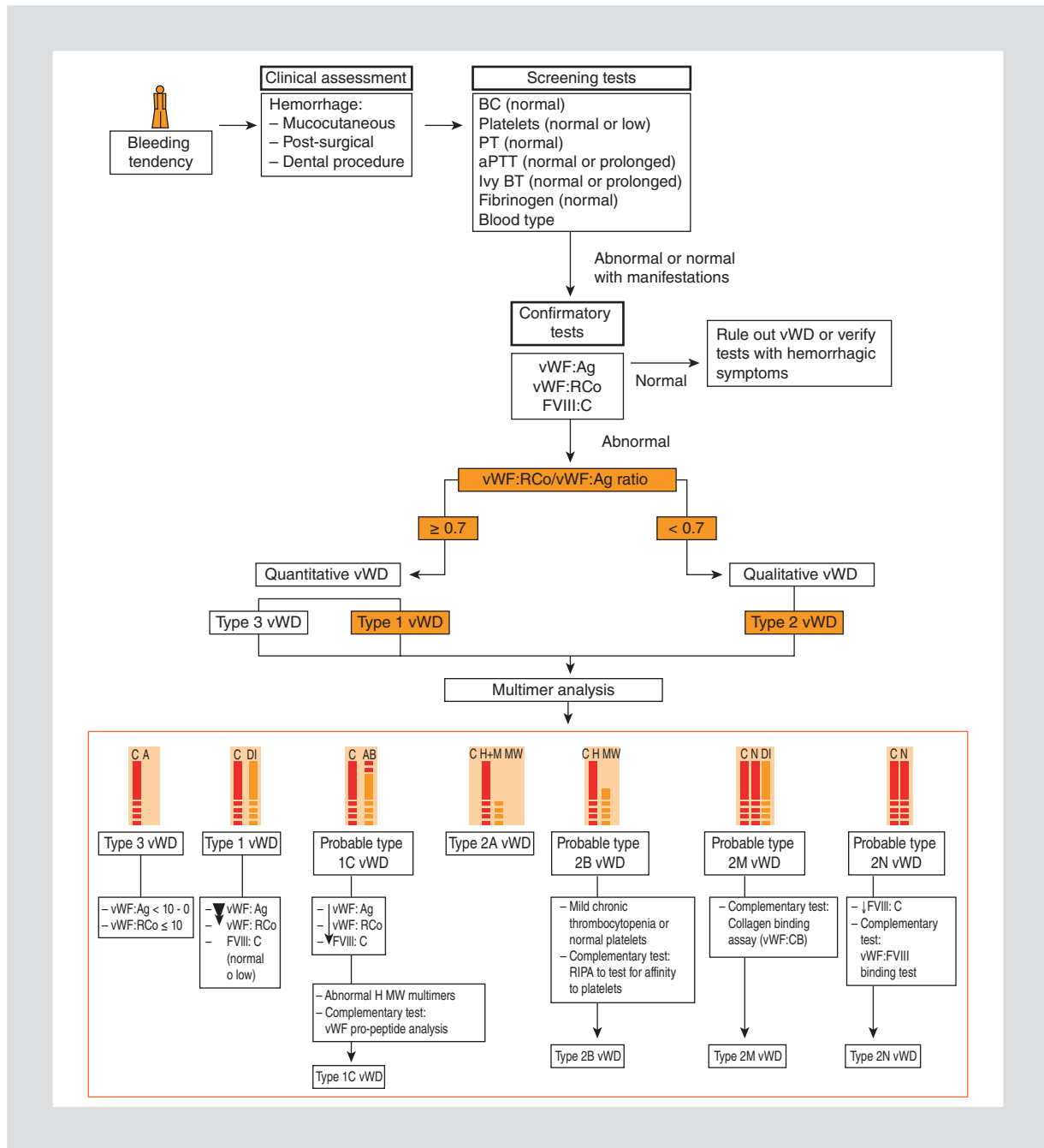
### **Complementary tests for diagnosis**

Complementary tests for vWD subtypes confirmation are essential in the diagnostic strategy (thus showing that the multimer assay is far from being the gold standard). When a patient has a drastically decreased FVIII value (usually between 1 and 10%), prolonged activated partial thromboplastin time (aPTT) and hemarthrosis, there is probable presence of the 2N-type (Normandy) vWD variant, in which a binding defect between vWF and FVIII exists, but with normal multimer pattern<sup>4</sup>. Since this last finding is also characteristic of 2M-type vWD, the test is unable to discriminate between both variants<sup>4</sup>, and a clearly decreased FVIII:C value sets the standard to distinguish between both these subtypes.

Morales-De la Vega et al. (2008) identified some 2N-type vWD cases in a Mexican population previously diagnosed with mild hemophilia A<sup>5</sup>. They used the FVIII/vWF binding affinity test to confirm the diagnosis. The study population was comprised by 30 patients: 25 diagnosed with mild or moderate hemophilia A and 5 with suspected vWD. Three patients (~10%) with previous diagnosis of hemophilia A were reclassified as type 2N vWD.

vWD 2B has a multimer pattern lacking high molecular weight aggregates; however, due to variations in electroforetic scrolling, it can be mixed up with the 2A-subtype pattern. To confirm this variant, presence of mild thrombocytopenia should be considered and, in addition, the multimer analysis should be complemented with the ristocetin-induced platelet aggregation (RIPA) assay at very low concentration (< 0.6 mg/ml). Although usually there is no binding to platelets, in patients with vWD 2B characterized by mutations in the vWF platelet binding domain, there is increased affinity to the platelet receptor GP 1bA (glycoprotein 1b $\alpha$ ) (GP 1b $\alpha$ )<sup>2</sup>. Of note, pseudo-vWD or platelet-type vWD also shows increased affinity to ristocetin, but this is owing to mutations in GP 1ba, and shows a multimer pattern similar to that of vWD 2B; therefore, a crossover plasma assay is required to differentiate such variants and genetic diagnosis as final proof<sup>2</sup>.

The collagen binding (CB) assay measures vWF large multimers affinity towards type I or III collagens, and represents yet another way to measure functional activity of the molecule without the use of ristocetin. The test is useful to differentiate type 2M from mild type 1 vWD, both with normal multimeric patterns and CB, but with decreased vWF:RCo/vWF:Ag ratio (< 0.7) only in 2M vWD.



**Figure 1.** vWD diagnostic algorithm. It is advisable to use a control (C) for the multimer analysis with a pool of plasma according to the patient's blood-type. In type 1 vWD, vWF:Ag and vWF:RCo values can range from normal (mild type 1 vWD) to very decreased (severe type 1 vWD). N: normal multimer pattern; DI: decreased intensity (mild to severe) of multimer pattern; A: complete absence of multimers; AB: abnormal high molecular weight multimers; H + M MW: absence of high and medium molecular weight multimers; H MW: absence of high molecular weight multimers.

The vWF pro-peptide analysis allows for the rate at which vWF is cleared from blood circulation to be known. Branchford et al.<sup>3</sup> point out that vWF rapid elimination from circulation, secondary to mutations in type 1 vWD, has therapeutic implications, since these patients show an initial vigorous response; however,

vWF blood concentration tends to abruptly drop in a 3-h period. These patients can be identified when normal synthesized vWF:Ag and pro-peptide ratio (1:1) is altered in favor of the pro-peptide, which indicates an increased vWF clearance and allows for type 1 C vWD or Vicenza-type to be diagnosed<sup>3</sup>.

## Genetic diagnosis

vWD genetic diagnosis is complex due to the difficulty to identify the causative mutation in the *vWF* gene, which measures 178 kb and contains 52 exons; in addition, the presence of a highly polymorphic pseudo-gene in chromosome 22 can introduce confusion when obtaining and interpreting the sequencing data<sup>3</sup>. Since mutations and SNPs not only affect the amounts of vWF, but also its function and CB domains, platelets, endothelium, FVIII:C, etc., this explains the broad pleiotropic effect on the phenotype, which has determined for genetic diagnosis to be essentially performed as basic research and in a very restricted way for management purposes<sup>3</sup>.

In order to optimize the diagnosis, we should consider that exons 18, 19, 20, 28, 45 and 52 of the *vWF* gene are sites prone to present mutations<sup>1</sup>; therefore, the molecular diagnosis can initially focus on these regions. Another strategy is the directed search for mutations by specific domain, according to clinical and laboratory diagnosis and based on the vWD subtype that might indicate the protein domains involved. This way, we will be able to provide rapid and accurate genetic counseling at a lower cost.

Some cases diagnosed with the previously described screening require additional genetic diagnosis, such as when the 2B vWD variant is trying to be differentiated from platelet-type vWD, or when the diagnosis is required to be confirmed, as with Normandy 2-type vWD<sup>3</sup>. Furthermore, compound heterozygous patients

can be found, with different subtype mutations difficulting protein analysis tests interpretation.

With no doubt, efforts in conducting the *vWF* gene analysis have yielded a large number of mutations and polymorphisms that have been highly useful to partially understand the vWF complex function and structure. With the above considerations, and according to the complex clinical and laboratory approach required for vWD diagnosis, the diagnostic algorithm illustrated in figure 1 is suggested.

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