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

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Dear Mr. Editor

The present letter is intended to clarify some points with regard to the review article entitled "Diagnostic algorithm for von Willebrand Disease (vWD) in a Mexican population" (Gac Med Mex. 2015;151:399-402), since we consider it has some important deficiencies that might mislead the reader; they are summarized as follows:

1. The recommendations for the diagnosis of this disease are international and not only for the Mexican population.

2. The abbreviations FvW:RCo and FvW:CB are a combination of Spanish and English, which is inappropiate. The internationally accepted abbreviations should be used instead: VWF:RCo and VWF:CB.

3. The term von Willebrand factor (VWF) *aggregates* is incorrect; the appropriate one is VWF *multimers.*

4. The review mentions that clearly decreased FVIII:C levels set the standard to distinguish between VWD 2M and 2N subtypes. However, FVIII levels do not set the standard to distinguish both subtypes, since the results of the VWF:RCo and RIPA tests, as well as that of the VWF:RCo/VWF:Ag ratio are also different between both subtypes of the disease. Moreover, in some cases, the VWF:CB test and the VWF:CB/VWF:Ag ratio are abnormal for the VWD 2M subtype, which is an additional difference with the VWD 2N subtype.

5. The review suggests using ristocetin at very low concentration (< 6 mg/ml) in the RIPA test to distinguish between the VWD 2A and 2B subtypes, which is ambiguous. The literature recommends using ristocetin concentrations of 0.5 mg/ml or even lower.

6. The review mentions that the VWF:CB test measures large VWF multimers affinity with type I, III or IV collagen. This is inaccurate. The VWF:CB test does not measure binding to type IV collagen, since currently recommended tests contain only a mixture of type I (95%) and type III (5%) collagens.

7. The article mentions that the VWF:CB test is useful to differentiate type 2M VWD from mild type 1, both with multimeric patterrns and normal VWF:CB, but decreased VWF:RCo/VWF:Ag ratio (< 0.7) only in 2M VWD. Today we know that type 2M VWD is also characterized by alterations in the collagen-binding domain. Therefore, patients with 2M VWD can show abnormal results in the VWF:CB test and normal results for the VWF:RCo/VWF:Ag ratio (≥ 0.7).

8. The diagnostic algorithm (DA) considers blood type as a screening test to diagnose VWD, which is incorrect, since its knowledge does not inform on the hemostatic status of a patient.

9. The DA proposes that blood count is normal in VWD. However, this is not useful for all patients, since some of them have anemia or sometimes pseudothrombocytopenia, as in type 2B VWD.

10. The DA suggests that VWF:Ag or FVIII normal results rule out VWD. However, this does not occur in type 2 VWD, since the results of the VWF:Ag and FVIII tests can be normal.

11. The DA states that in type 3 VWD, VWF:Ag = 0 and VWF:RCo ≤ 10 U/dl, which is completely illogical: how can there be ristocetin activity if there is no VWF? Actually, VWF:Ag should be < 4.

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12. The DA establishes that VWF:Ag or FVIII normal results rule out VWD. However, this does not occur in VWD 2A, 2B and 2M subtypes, since in these cases, the results can be normal.

13. The DA suggests performing the "vWF:FVIII:C" test in 2N type VWD, but this test does not exist. The correct test is VWF:FVIIIB, which measures VWF binding capacity to FVIII. The FVIII:C abbreviation refers to the functional test that measures FVIII activity.

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Dear Mr. Editor:

We thank our colleagues Jesús Hernández-Juárez, Manuel Moreno-Hernández and Abraham Majluf-Cruz for their observations to our brief review "Diagnostic algorithm for von Willebrand Disease (vWD) in a Mexican population" (Gac Med Mex. 2015;151:399-402). Although we acknowledge some errors that we failed to opportunely detect (especially in the figure), we also found that some observations are completely inadequate and, below, we answer all the questions and comments expresed by the authors (*in italics*):

1. The recommendations for the diagnosis of this disease are international and not only for the Mexican Population.

We believe the comment is inadequate, since we are not talking about "exclusive" recommendations for the Mexican population, but focused on determinate characteristics of Mexicans that we consider important pointing out, as it remains clear in the 2nd paragraph of p. 400. Thus, our review is intended to integrate international recommendations and the experience of recent national works to propose a von Willebrand Disease (vWD) diagnosis algorithm (DA) focused on the characteristics of our population.

For example, since blood type O causes a 20-25% plasma von Willebrand Factor (vWF) decrease[1,2], and given that this group is predominant in our population, according to Melo-Nava et al. 2007 (reference no. 1 of our review), we underscore that a low vWF plasma concentration can mask a mild quantitative vWD. Such particularity has not been previously highlighted in the recommendations for the diagnosis of vWD in our population and marks the originality of our work.

2. The abbreviations FvW:RCo and FvW:CB are a combination of Spanish and English, which is inappropiate. The internationally accepted abbreviations should be used instead: VWF:RCo and VWF:CB.

Although we agree on the use of internationally standardized abbreviations, we consider the used abbreviations to be adequate because this is a publication in the Spanish language and because they were accepted by the Editorial Committee. In addition, they meet the consistency requirement and are defined when quoted for the first time in the body of the text.

With regard to the Spanish-English combination, we emphasize that we were based on standard abbreviations in our country used in other publications in Spanish – surely known by the authors of the letter –, such as the *Guía de práctica clínica de diagnóstico y tratamiento de la enfermedad de von Willebrand* of the Ministry of Health (FvW:RCo on p. 14 and FvW:CB on p. 22)[1], the study by Morales-De la Vega et al. of 2008 (FvW:RCo on p. 57), quoted in our review (reference no. 5), and the *Guía rápida de la enfermedad de von Willebrand*, by Martínez-Murillo 2011[2], that employs different nomenclatures for FvW:RCo, but none as the suggested one (FvW:Ricof on p. 17; FvW:RICOF on box 5.2 at p. 30; FvW:RiCo on p. 31; FvW:RCo on box 5.4 at p. 31 and FvW:CB on p. 54).

3. The term von Willebrand factor (VWF) aggregates is incorrect; the appropriate one is VWF multimers.

The comment diverges from what we refer in the text, since in no paragraph we use the expression vWF *aggregates*, but be refer to vWF multimer aggregates; to avoid repetitions in some sentences (1st paragraph, 2nd column, antepenultimate line, p. 400 and 1st paragraph, 1st column, below the figure, p. 401). Moreover, the molecular weight of a multimeric complex such as vWF is in agreement with the number of multimers that form "aggregates" of different quantities.

4a. The review mentions that clearly decreased FVIII:C levels set the standard to distinguish between VWD subtypes 2M and 2N. However, FVIII levels do not set the standard to distinguish both subtypes, since the results of the VWF:RCo and RIPA tests, as well as that of the VWF:RCo/VWF:Ag ratio are also different between both subtypes of the disease.

Since the 2M and 2N variants have a normal multimer pattern, FVIII:C drastic decrease is what characterizes the 2N variant and, in fact, it is the basis of its etiology, as textually referred by Favaloro 2011 (reference no. 2 of our review): "2N vWD nas an inherent defect in vWF that causes the defective binding to FVIII. Therefore, plasma FVIII is labile, prone to proteolysis and FVIII:C tends to be lower than vWF (with diminished relationships between FVIII/vWF being evident)" (subsection 4 on 2N vWD, p. 557).

It is well known that 2N vWD, in addition to the similarity of the clinical phenotype, can be confused and be wrongly diagnosed as moderate hemophilia A due to a significant FVIII:C decrease. This is conclusively shown in the study of Mexican patients by Morales-De la Vega et al. 2008 (reference no. 5 of our review) and Favaloro 2011 (reference no. 2 of our review).

Thus, on the initial paragraph of subsection "Complementary tests for diagnosis" (p. 400) we specify that clearly decreased FVIII:C sets the standard to distinguish the 2N subtype, which must be verified with the vWF:FVIII binding test.

The correct observation of the authors on the vWF:RCo/vWF:Ag ratio differing between both variants led us to correct the figure we show in this reply. However, since the vWF:RCo and vWF:Ag values can be highly variable and not definitory for 2N, we insist on that the only test that allows for its confirmation is the vWF:FVIII binding assay.

4b. Moreover, in some cases, the VWF:CB test and the VWF:CB/VWF:Ag ratio are abnormal for the VWD 2M subtype, which is an additional difference with the VWD 2N subtype.

We encourage the authors to examine the information more carefully, since their claim only applies to very rare cases of 2M vWD. According to the article by Favaloro 2007 (quoted by the authors of the letter, reference no. 4), and especially to box 1 on which surely they base their comment (p. 732), the 2M and 2N variants differ in the above quoted parameters. However, the authors of the letter should examine the box footnote, as well as the text, which indicates that this does not apply for the 2M variant, but rather what "is generally true" (p. 740), i.e., that in most cases, 2M has dysfunctional platelet binding activity, a decreased vWF:RCo/vWF:Ag ratio and a normal vWF:CB/vWF:Ag ratio, similar to subtype 2N. The latter ratio is altered in 2A and, therefore, in any case, it would be a discriminatory criterion between the 2A and 2M variants and with regard to type 1 vWD, as we indicate in the review (2nd paragraph, 1st column, p. 402).

Since both parameters are generally similar in 2M and 2N, we insist on that what sets the discriminatory standard in 2N is the FVIII decrease, and hence the recommendation of performing the vWF:FVIII specific binding test for confirmation, which we also indicate in our review, consistent with Favaloro 2011 (reference no. 2 of our review) and Favaloro 2007, quoted by the authors of the letter (reference no. 4).

5. The review suggests using ristocetin at very low concentration (< 6 mg/ml) in the RIPA test to distinguish between VWD 2A and 2B subtypes, which is ambiguous. The literature recommends using ristocetin concentrations of 0.5 mg/ml or even lower.

We agree with the authors. The article has a mistake (paragraph, 2nd column, p. 401), since the concentration in the RIPA assay to distinguish between vWD 2A and 2B subtypes should be < 0.6 mg/ml. We know this information and have recommended it in the study of our patients; however, we didn't notice that we ommited the decimal point.

6. The review mentions that the VWF:CB test measures large VWF multimers affinity with type I, III or IV collagen. This is inaccurate. The VWF:CB test does not measure binding to type IV collagen, since currently recommended tests contain only a mixture of type I (95%) and type III (5%) collagens.

We recognize that this is also our mistake (2nd paragraph, 1st column, p. 402), since the collagen binding test only includes types I and III.

7. The article mentions that the VWF:CB test is useful to differentiate type 2M VWD from mild type 1, both with normal multimeric patterrns and VWF:CB, but a decreased VWF:RCo/VWF:Ag ratio (< 0.7) only in 2M VWD. Today we know that type 2M VWD is also characterized by alterations in the collagen-binding domain. Therefore, patients with 2M VWD can show abnormal results in the VWF:CB test and normal results for the VWF:RCo/VWF:Ag ratio (≥ 0.7).

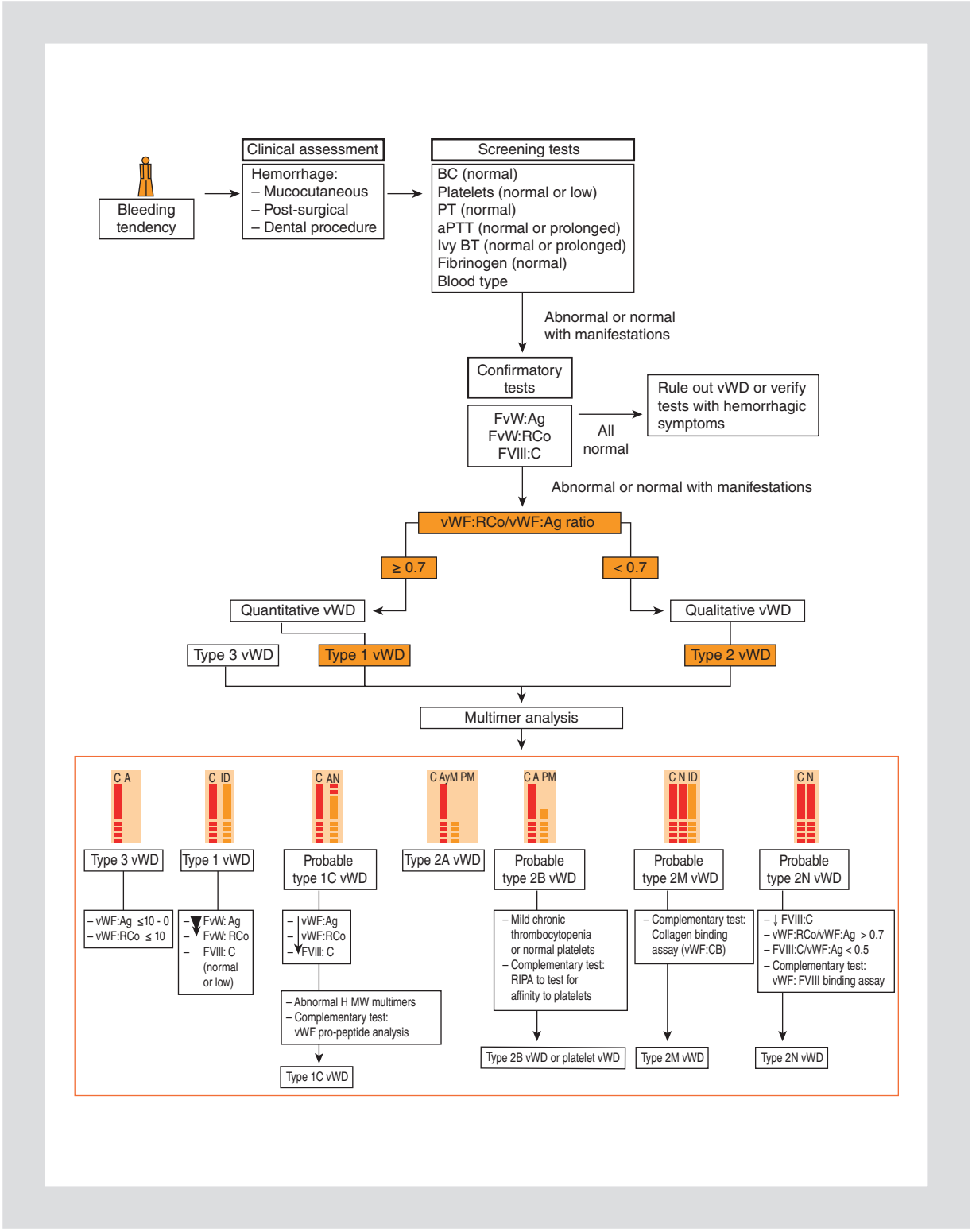

Figure 1. *vWD diagnostic algorithm.*
It is advisable to use a control (C) for the multimer analysis with a pool of plasma according to the patient's blood type. In type 1 vWD, vWF:Ag and vWF:RCo values can range from normal (mild type 1 vWD) to very decreased (serious type 1 vWD) and absent (type 3 vWD). Type 2 vWD groups a series of vWF functional defects in the interaction with collagen (2A vWD), with GP1bA (2B vWD) or with FVIII (2N vWD), as well as vWF with non-evident AB by multimer analysis, with altered binding to platelets without affecting collagen binding (2M vWD). N: normal multimer pattern; DI: multimeter pattern decreased intensity (mild to serious); A: complete abscence of multimers; AB: abnormal high molecular weight multimers; H + M MW: abscence of high and medium molecular weight multimers; H MW: absence of high molecular weight multimers.

As previously stated in point 4, this is true only for 2M exceptional cases. According to Favoloro 2007 (quoted by the authors of the letter) and Favoloro 2011 (reference no. 2 of our review), the rare variant the authors of the letter refer, also known as 2CB variant, shows decreased activity to type I and III collagen, while the multimer pattern, the vWF:RCo/vWF:Ag ratio and RIPA are normal[6,7].

8. The diagnostic algorithm (DA) considers blood type as a screening test to diagnose VWD, which is incorrect, since its knowledge does not inform on the hemostatic status of a patient.

We understand that this remains a subject of debate within the community of hematologists. Anyway, after carefully analyzing the algorithm, we decided to include the blood type in the screening tests intentionally and on good grounds. As indicated in our review, blood type accounts for a 20-25% variation in vWF plasma concentration, and this in turn determines an individual's hemostatic status[3]. Therefore, we reiterate that blood type is a useful test on vWD diagnosis and that it is also essential to interpret antigen levels and vWF activity on each patient. In addition, blood type has been shown to be determinant on hemorrhagic tendency[2-4].

9. The DA proposes that blood count is normal in VWD. However, this is not useful for all patients, since some of them have anemia or sometimes pseudothrombocytopenia, as in type 2B VWD.

The comment does not apply, since thrombocytopenia is included in the DA figure. Anemia is not a vWD diagnostic criterion, but may result from recurrent, uncontrolled hemorrhages.

10. The DA suggests that VWF:Ag or FVIII normal results rule out VWD. However, this does not occur in type 2 VWD, since the results of the VWF:Ag and FVIII tests can be normal.

Although not completely in agreement, we accept that the figure might suggest what the observation refers and, therefore, we propose modifying it to indicate that normal values of the vWF:Ag **+** vWF:RCo **+** FVIII parameters (all concurrently) rule out vWD, provided such results are verified on separate occasions and there are no hemorrhagic symptoms. If there is hemorrhagic tendency, regardless of normal confirmatory parameters, there may be some coagulopathy or mild quantitative vWD, and testing should be further continued.

11. The DA states that in type 3 VWD, VWF:Ag = 0 and VWF:RCo ≤ 10 U/dl, which is completely illogical: how can there be ristocetin activity if there is no VWD? Actually, VWF:Ag should be < 10.

Indeed, type 3 vWD is characterized by indetectable levels of vWF:AG and vWF:RCo < 10 U/dl or indetectable levels; therefore, it is necessary to specify that the vWF:Ag value can drop between 10 and 0, emphasizinig on the higher resolution of the antigen test by ELISA and with verification of vWF multimers absolute absence. However, it should be noted that putting it this way was intentional (although it may sound illogical) in order to highlight the inaccuracy of the vWF:RCo test by plate-platelet agglutination, which has limited sensitivity, specificity and reproducibility, as referred by Favaloro 2011 and Majluf-Cruz et al. 2013 (references no. 2 and 4, respectively, of our review).

12. The DA establishes that VWF:Ag or FVIII normal results rule out VWD. However, this does not occur in VWD 2A, 2B and 2M subtypes, since in these cases, the results can be normal.

As in point 9, it is a biased interpretation of the information, since it is incorrect indicating that the DA figure implies such claim. However, we accept that the figure can be misinterpreted and we consider that we should clarify this point by indicating, in the legend after screening, that the results of the confirmatory tests combined (vWF:Ag + vWF:RCo + FVIII:C) can be "abnormal or normal with manifestations".

We disagree on the information being inadequate, since in all described cases, 2A, 2B and 2M, vWF:Ag is generally decreased according to multiple works[1,2], such as Favaloro 2007 (quoted by the authors of the letter) and Favaloro 2011 (reference no. 2 of our review); now, a normal vWF:Ag value forces to consider the blood type. In summary, our intention was to highlight some specific aspects in order to improve vWD diagnostic accuracy, with special emphasis on the Mexican population, as well as on available tests in Mexico.

13. The DA suggests performing the "vWF:FVIII:C" test in 2N type VWD, but this test does not exist. The correct test is VWF:FVIIIB, which measures VWF binding capacity to FVIII. The FVIII:C abbreviation refers to the functional test that measures FVIII activity.

In the last paragraph of p. 400 of the article we refer to the vWF:FVIII binding test, and this way should have appeared in the figure; evidently, it was a typing error,

and we are not proposing a "non-existing" test. We have corrected this mistake in the figure we have asked to be added to this reply with all previously mentioned modifications.

The observation on the vWF:FVIII binding affinity test (reference no. 5 of the review) being a "non-existing" test and clarifying to us the meaning of FVIII:C, which we do define in our review (2nd paragraph, 1st column, section "Diagnosis by screening and confirmatory tests"), appears to us to be in the verge of sarcasm and it constitutes a lack of respect and an unethical attitude by the authors of the letter.

Moreover, the vWF/FVIII:C test would not be that "non-existing" since, a few years ago, vWF and FVIII binding assessment included the measurement of FVIII not bound to vWF by coagulometric methods[5]. Similarly, Martínez Murillo, expert on the area and co-author of the Mexican study that identified three patients with 2N vWD by means of the above-mentioned vWF:FVIII binding assay, employs the "vWF/FVIII:C" notation to indicate the binding site of vWF with FVIII (figure 5.3, p. 32; figure 5.1 footnote, p. 28)[2].

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