

## FORUM

# 2B von Willebrand disease diagnosis: Considerations reflecting on 2021 multisociety guidelines

Maha Othman MD, PhD<sup>1,2</sup>   | Emmanuel J. Favaloro PhD<sup>3,4,5</sup> 

<sup>1</sup>Department of Biomedical and Molecular Sciences, School of Medicine, Queen's University, Kingston, Ontario, Canada

<sup>2</sup>School of Baccalaureate Nursing, St Lawrence College, Kingston, Ontario, Canada

<sup>3</sup>Department of Haematology, Institute of Clinical Pathology and Medical Research (ICPMR), NSW Health Pathology, Westmead Hospital, Westmead, New South Wales, Australia

<sup>4</sup>Sydney Centres for Thrombosis and Haemostasis, Westmead, New South Wales, Australia

<sup>5</sup>School of Biomedical Sciences, Charles Sturt University, Wagga Wagga, New South Wales, Australia

## Correspondence

Maha Othman, Department of Biomedical and Molecular Sciences, School of Medicine, Queen's University, Bottrell Hall, 18 Stuart Street, Kingston, Ontario K7L 3N6, Canada.  
Email: othman@queensu.ca

**Handling Editor:** Dr Johnny Mahlangu

## Abstract

The recent American Society of Hematology/ISTH/National Hemophilia Foundation/World Federation of Hemophilia 2021 guidelines on the diagnosis of von Willebrand disease (VWD) is an outstanding effort to unify the diagnosis of VWD. However, as mentioned in the guidelines, there are limitations due to the low certainty in the evidence identified for most questions. The panel encouraged critical review of the guidelines. Compared to other subtypes, there is considerable complexity with diagnosis of type 2B VWD, a type that results from a gain-of-function mutation in the VWF gene. Additionally, the discrimination from its phenocopy platelet-type VWD, representing a gain-of-function mutation in the *GP1BA* gene, is crucial as this determines treatment decisions. In this forum, we highlight the complexities of a type 2B VWD diagnosis; discuss important issues with respect to these complexities: genotype/phenotype/clinical correlations, challenges with platelet aggregation and ristocetin-induced platelet agglutination testing, platelet count, and thrombocytopeny; and, finally, suggest the consideration of some of these complexities in future iterations of the VWD guidelines.

## KEYWORDS

2B VWD, platelet aggregation, thrombocytopeny, VWD guidelines, VWF

## 1 | INTRODUCTION

The recent American Society of Hematology/ISTH/National Hemophilia Foundation/World Federation of Hemophilia 2021 guidelines on the diagnosis<sup>1</sup> of von Willebrand disease (VWD) are undoubtedly a step forward in unifying the diagnosis of this common and important bleeding disorder. The first key effort in the classification and diagnosis was made by Sadler et al<sup>2</sup> in 2006, and the last key publications before the new guidelines were over 7 years ago now.<sup>3,4</sup> Indeed, as mentioned in the guidelines, there are limitations due to the low certainty in the evidence identified for most questions. We

are pleased to see the panel encouraging critical review of the guidelines, which may facilitate regular revision/update.

Compared to types 1 and 3, the diagnosis of type 2 VWD subtypes can be particularly challenging.<sup>5</sup> Type 2B VWD represents ≈5% of all VWD types.<sup>6</sup> The accurate diagnosis of this subtype is critical for many reasons, including concerns or potential harms related to the use of desmopressin (DDAVP).<sup>7</sup> A recent study showed, in a retrospective and prospective reassessment, the diagnosis of type 2B VWD was confirmed only in 60% to 77% of cases with a preexisting diagnosis.<sup>8</sup> Two main factors contribute to the variability in diagnosis are laboratory variations and atypical presentations.<sup>9</sup>

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2021 The Authors. *Research and Practice in Thrombosis and Haemostasis* published by Wiley Periodicals LLC on behalf of International Society on Thrombosis and Haemostasis (ISTH).

In this forum, we discuss 2 main recommendations<sup>9,10</sup> of the diagnostic guidelines.<sup>1</sup> The panel “suggests either VWF multimer analysis or VWF collagen binding/VWF antigen (VWF:Ag) (the ratio of VWF collagen binding to antigen) to diagnose type 2 VWD for patients suspected of type 2A, 2B, or 2M in need of additional testing” and “suggests targeted genetic testing over low-dose ristocetin-induced platelet agglutination (RIPA) to diagnose type 2B VWD for patients suspected of type 2A or 2B in need of additional testing.” Additionally, in the diagnosis algorithm, it was suggested that “patients suspected to have 2B shall undergo genetic testing and upon finding a type 2B genetic variation they are then confirmed 2B.”

Type 2B is a qualitative defect that results from a gain-of-function mutation in A1 domain of the VWF gene, leading to increased binding affinity of plasma VWF for platelet glycoprotein (GP) 1b $\alpha$ , loss of high-molecular-weight multimers (HMWMs), thrombocytopenia, and bleeding. Compared to other subtypes, there is considerable complexity with diagnosis of type 2B VWD. Additionally, the discrimination from its phenocopy platelet-type (PT)-VWD, which results from a gain-of-function mutation in the GP1BA gene, is crucial as this determines treatment decisions. Here, we highlight/discuss the reasons for those complexities and suggest consideration of some of these complexities in future iterations of the guidelines (Figure 1).

## 2 | COMPLEXITIES WITH LAB DIAGNOSIS—PARTICULARLY RIPA TESTS AND POOR AWARENESS

The laboratory suspicion of type 2B VWD usually starts with an abnormal initial VWD screen with ratio of platelet-dependent VWF activity/VWF:Ag ratio <0.7 (as listed in recommendation 8)<sup>1</sup> together with evidence of enhanced VWF platelet binding (historically known as ristocetin-induced platelet agglutination (or aggregation; RIPA)

test in response to 0.5 mg/mL of ristocetin). This test is also positive in the disease phenocopy PT-VWD.<sup>10</sup>

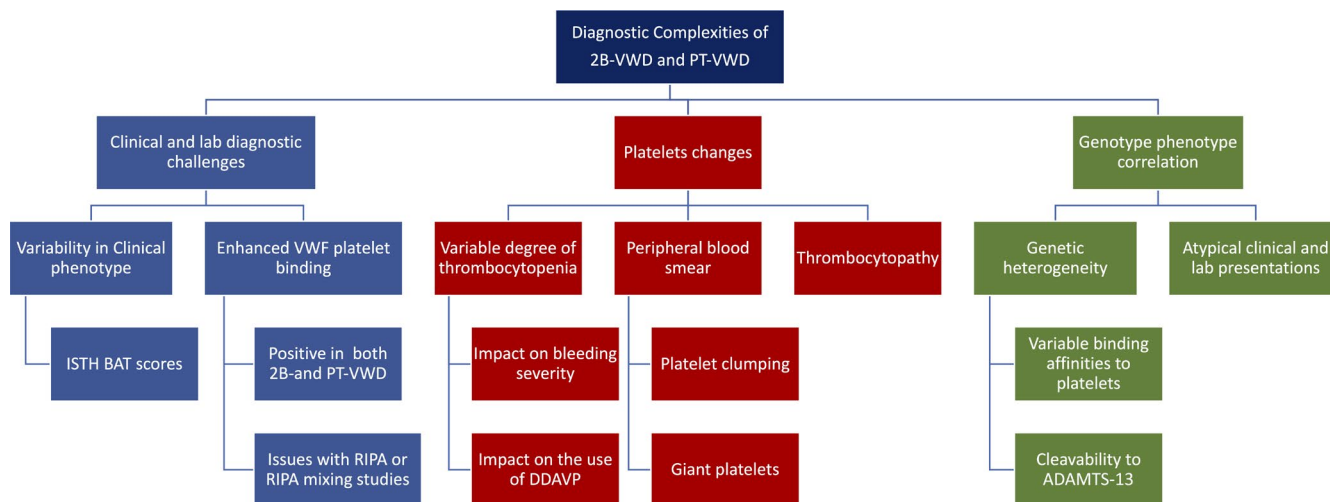
Over the years, RIPA has further developed into the more robust assessment of “RIPA mixing studies,” where mixing each of the plasma and platelets or platelet-rich plasma from the patient with those of the normal control can identify the origin of the defect on either side of the interaction. Such modified tests have been well described<sup>11</sup> and have the advantage of differentiating simultaneously between 2B and its phenocopy PT-VWD.<sup>11-13</sup> This has proven its validity when compared with genetic testing and is useful in thrombocytopenic patients in whom RIPA testing is difficult to assess. The test is also included in the ISTH diagnostic algorithm for PT-VWD.<sup>14</sup>

We believe the issues with RIPA or RIPA mixing studies are not so much in the validity but rather in (A) poor applicability/challenges among lab personnel, especially in cases of low platelet count; (B) limited awareness of the RIPA mixing test,<sup>10-12</sup> its value and its interpretation, among lab personnel and/or physicians; (C) the different commercial sources of ristocetin; and (D) the cut of value used to consider a positive test.

We also recognize those limitations are barriers to the effective utilization of RIPA mixing studies in making the correct diagnosis. We recommend when RIPA is not feasible, to consider either flow cytometry or to conduct the genetic sequence analysis to confirm the diagnosis.

## 3 | COMPLEXITIES RELATED TO PLATELET EXAMINATION AND ITS IMPACT ON DIAGNOSIS OF BOTH 2B AND PT-VWD

A variable degree of thrombocytopenia is often observed in patients with either type 2B or PT-VWD.<sup>15</sup> Occasionally and in some variants, platelet clumping and/or giant platelets are also seen in a peripheral blood smear.<sup>16,17</sup> This underscores the importance of platelet



**FIGURE 1** Complexities of type 2B- and PT-VWD diagnosis. BAT, Bleeding Assessment Tool; PT, platelet-type; RIPA, ristocetin-induced platelet agglutination; VWD, von Willebrand disease

count and morphology in the diagnostic process. While the low platelet count complements other diagnostic elements, this variation also determines the severity of the clinical phenotype (mild to severe bleeding phenotype) and thus the ISTH Bleeding Assessment Tool (BAT) score,<sup>15</sup> which is used as an objective tool in diagnosis of VWD. More importantly, it also determines the use of DDAVP.<sup>7</sup> The assessment of platelets in type 2B and PT-VWD patients is often underappreciated and receives much less awareness among physicians and specialists.

Thrombocytopathy (alteration of platelet  $\alpha$ IIb $\beta$ 3 engagement and defective signaling) has been described recently in patients with type 2B VWD with the common genetic variant p.V1316 M.<sup>18</sup> This is associated with a severe bleeding phenotype with ISTH BAT of 14; atypical in type 2B VWD. Whether the platelet dysfunction observed with p.V1316 M is also found in other variants, is currently unknown, but impacts the clinical presentation and management. In this, it is worth noting the potential use of thrombopoietin receptor agonists as an alternative therapeutic approach in cases of VWD type 2B associated with the p.V1316 M mutation and severe thrombocytopenia.<sup>19</sup> The intrinsic platelet abnormality has been also discussed by others.<sup>20</sup> Thrombocytopathy has also been described in PT-VWD (both patients and mouse model),<sup>21,22</sup> The above information indicates that platelet examination needs to be considered in the diagnosis of both phenocopies of the disease and that much awareness about this is required.

#### 4 | COMPLEXITIES RELATED TO GENETIC VARIATIONS AND GENOTYPE-PHENOTYPE CORRELATION

The heterogeneity in clinical presentation and bleeding severity of type 2B VWD is evident in literature.<sup>23</sup> Clinical severity is often dictated by the genetic variations, which are also highly diverse. Atypical cases (clinical and lab including atypicality in loss of HMWM or in RIPA) have been described, raising challenging questions regarding phenotype-genotype correlation and even the certainty of phenotype-genotype assignment.

The location and biochemical nature of 2B mutations and their effects can be complex. The VWF A1 domain spans 1260 to 1479 aa of the VWF protein and is coded for by exon 28 of the VWF gene. GPIIb binds VWF within aa 1244 to 1481; within or in close proximity to a cys disulfide loop 1272 to 1458.<sup>24</sup> Functional studies have shown that different mutations at different residues, and sometimes different amino acid substitutions at the same residue, can result in variable binding affinities to platelets and also variable phenotype. Mutations just outside of the A1 loop can also induce A1 loop-dependent VWF/platelet binding and yield a type 2B VWD phenotype. Additionally, various VWF mutations can affect the cleavability to ADAM-TS13,<sup>25,26</sup> resulting in a range of hemostatic phenotypes. For further details please refer to Othman and Favaloro.<sup>23</sup>

Several close proximity genetic variants (<http://www.vwf.group.shef.ac.uk/>) have been differently assigned to 2B and other types

such as 2A. While these assignments may be accurate, it is also possible that some 2A cases have been misdiagnosed, perhaps due to missing RIPA. Thus, relying on genetic test results to assign 2B accurately may be compromised.

Based on the above, we would request and hope that future iterations of the guidelines consider the complexities explained above. More specifically, we suggest the following:

1. Using a 2B VWD genetic variant as a confirmation of the diagnosis of 2B needs to be done alongside with an assessment of the clinical bleeding phenotype with careful platelet examination. This may positively influence diagnostic certainty.
2. RIPA mixing studies need to augment standard RIPA and be considered in the diagnostic algorithm of 2B-VWD and in the differentiating from PT-VWD. Alongside this, raising international awareness about the testing steps, applicability, and interpretation, especially in cases of low platelet count (often encountered in both type 2B and PT-VWD), would be of utmost importance. This shall help reduce the variability in lab diagnosis.
3. The complete blood count proposed early in the VWD diagnosis, may need to be repeated, when and if type 2B/PT-VWD are suspected with particular attention given to further platelet assessments (count and morphology) to aid diagnosis.

#### RELATIONSHIP DISCLOSURE

Authors have nothing to disclose.

#### AUTHOR CONTRIBUTIONS

Both MO and EJF designed the concept. MO drafted the manuscript. EJF evaluated and critically analyzed the information and reviewed the manuscript.

#### ORCID

Maha Othman  <https://orcid.org/0000-0001-7562-203X>

Emmanuel J. Favaloro  <https://orcid.org/0000-0002-2103-1661>

#### TWITTER

Maha Othman  @MahaOthman8

#### REFERENCES

1. James PD, Connell NT, Ameer B, et al. ASH ISTH NHF WFH 2021 guidelines on the diagnosis of von Willebrand disease. *Blood Adv.* 2021;5(1):280-300.
2. Sadler JE, Budde U, Eikenboom JC, et al.; Working Party on von Willebrand Disease Classification. Update on the pathophysiology and classification of von Willebrand disease: a report of the Subcommittee on von Willebrand factor. *J Thromb Haemost.* 2006;4:2103-2114.
3. Laffan MA, Lester W, O'Donnell JS, et al. The diagnosis and management of von Willebrand disease: a United Kingdom Haemophilia Centre Doctors Organization guideline approved by the British Committee for Standards in Haematology. *Br J Haematol.* 2014;167(4):453-465.

4. Nichols WL, Hultin MB, James AH, et al. von Willebrand disease (VWD): evidence-based diagnosis and management guidelines, the National Heart, Lung, and Blood Institute (NHLBI) Expert Panel report (USA). *Haemophilia*. 2008;14(2):171-232.
5. Favalaro EJ, Dean E, Arunachalam S, Vong R, Mohammed S. Evaluating errors in the laboratory identification of von Willebrand disease (VWD) using contemporary von Willebrand factor (VWF) assays. *Pathology*. 2021;S0031-3025(21)00433-5. doi:10.1016/j.pathol.2021.07.001
6. Favalaro EJ. Von Willebrand disease: local diagnosis and management of a globally distributed bleeding disorder. *Semin Thromb Hemost*. 2011;37(5):440-455.
7. Kruse-Jarres R, Johnsen JM. How I treat type 2B von Willebrand disease. *Blood*. 2018;131(12):1292-1300. doi:10.1182/blood-2018-04-844357
8. DiGiandomenico S, Christopherson PA, Haberichter SL, Abshire TC, Montgomery RR, Flood VH. Zimmerman Program Investigators. Laboratory variability in the diagnosis of type 2 VWD variants. *J Thromb Haemost*. 2021;19(1):131-138.
9. Mikhail S, Aldin ES, Streiff M, Zeidan A. An update on type 2B von Willebrand disease. *Expert Rev Hematol*. 2014;7(2):217-231.
10. Othman M. Platelet-type Von Willebrand disease: three decades in the life of a rare bleeding disorder. *Blood Rev*. 2011;25(4):147-153.
11. Fronthoff JP, Favalaro EJ. Ristocetin-induced platelet aggregation (RIPA) and RIPA mixing studies. *Methods Mol Biol*. 2017;1646:473-494.
12. Favalaro EJ, Patterson D, Denholm A, et al. Differential identification of a rare form of platelet-type (pseudo-) von Willebrand disease (VWD) from Type 2B VWD using a simplified ristocetin-induced-platelet-agglutination mixing assay and confirmed by genetic analysis. *Br J Haematol*. 2007;139(4):623-626.
13. Favalaro EJ. Phenotypic identification of platelet-type von Willebrand disease and its discrimination from type 2B von Willebrand disease: a question of 2B or not 2B? A story of non-identical twins? Or two sides of a multidimensional or multifaceted primary-hemostasis coin? *Semin Thromb Hemost*. 2008;34(1):113-127.
14. Othman M, Greslele P. Guidance on the diagnosis and management of platelet-type von Willebrand disease: a communication from the platelet physiology subcommittee of the ISTH. *J Thromb Haemost*. 2020;18(8):1855-1858.
15. Kaur H, Ozelo M, Scovil S, James PD, Othman M. Systematic analysis of bleeding phenotype in PT-VWD compared to type 2B VWD using an electronic bleeding questionnaire. *Clin Appl Thromb Hemost*. 2014;20(8):765-771.
16. Nurden P, Chretien F, Poujol C, Winckler J, Borel-Derlon A, Nurden A. Platelet ultrastructural abnormalities in three patients with type 2B von Willebrand disease. *Br J Haematol*. 2000;110(3):704-714.
17. Slayton WB, Patel M, Sola-Visner M, et al. Type 2B von Willebrand disease associated with the release of platelet agglutinates from megakaryocytes in the bone marrow. *J Pediatr Hematol Oncol*. 2008;30(9):708-711.
18. Casari C, Berrou E, Lebret M, et al. von Willebrand factor mutation promotes thrombocytopathy by inhibiting integrin  $\alpha\text{IIb}\beta\text{3}$ . *J Clin Invest*. 2013;123(12):5071-5081.
19. Espitia O, Ternisien C, Agard C, Boisseau P, Denis CV, Fouassier M. Use of a thrombopoietin receptor agonist in von Willebrand disease type 2B (p.V1316M) with severe thrombocytopenia and intracranial hemorrhage. *Platelets*. 2017;28(5):518-520. doi: 10.1080/09537104.2016.1246717
20. Castaman G, Federici AB. Type 2B von Willebrand disease: a matter of plasma plus platelet abnormality. *Semin Thromb Hemost*. 2016;42(5):478-482.
21. Kaur H, Corscadden K, Ware J, Othman M. Thrombocytopathy leading to impaired in vivo haemostasis and thrombosis in platelet type von Willebrand disease. *Thromb Haemost*. 2017;117(03):543-555.
22. Bury L, Malara A, Momi S, Petit E, Balduini A, Greslele P. Mechanisms of thrombocytopenia in platelet-type von Willebrand disease. *Haematologica*. 2019;104(7):1473-1481.
23. Othman M, Favalaro EJ. Genetics of type 2B von Willebrand disease: "true 2B," "tricky 2B," or "not 2B." What are the modifiers of the phenotype? *Semin Thromb Hemost*. 2008;34(6):520-531.
24. Meyer D, Piétu G, Fressinaud E, Girma JP. Von Willebrand factor: structure and function. *Mayo Clin Proc*. 1991;66(5):516-523.
25. Ma Z, Su J, Zhang J, et al. The co-influence of VWD type 2B/2M mutations in the A1 domain and platelet GPIIb/IIIa on the rate of cleavage to VWF by ADAMTS13. *Thromb Res*. 2015;136(5):987-995.
26. Rayes J, Hommais A, Legendre P, et al. Effect of von Willebrand disease type 2B and type 2M mutations on the susceptibility of von Willebrand factor to ADAMTS-13. *J Thromb Haemost*. 2007;5(2):321-328.

**How to cite this article:** Othman M, Favalaro EJ. 2B von Willebrand disease diagnosis: Considerations reflecting on 2021 multisociety guidelines. *Res Pract Thromb Haemost*. 2021;5:e12635. doi:[10.1002/rth2.12635](https://doi.org/10.1002/rth2.12635)