Von Willebrand Disease: The Importance of Diagnostic Confidence

Von Willebrand disease (VWD) is the most common inherited bleeding disorder, found in approximately 3.2 million Americans, or 1% of the U.S. population. VWD is caused by either a defect or deficiency of Von Willebrand factor (VWF), an end-to-end polymerized glycoprotein that helps blood to clot. When a healthy individual sustains vascular injury, VWF promotes clotting by binding platelets to exposed collagen and shuttling factor VIII (FVIII) to injury sites to create fibrin clots.

As people with VWD either lack VWF or their VWF does not function properly, it takes longer for their blood to clot and puts them at risk of severe bleeding. Oftentimes, these individuals experience mucocutaneous bleeding, including heavy menstrual bleeding, epistaxis (nose bleeds), easy bruising, prolonged bleeding from minor injuries, gastrointestinal bleeding, and bleeding after dental work, childbirth and surgery. In rare instances, some patients experience joint and/or internal bleeding in severe cases. (James et al., 2021)

Because VWF levels vary among the population, providing an accurate VWD diagnosis can be especially challenging. Differing opinions on VWF reference ranges and inconsistent testing protocols among physicians create additional challenges when confirming a diagnosis.

Standardizing diagnostic testing increases confidence in diagnosis and leads to more impactful, timely treatment, improving outcomes and quality of life for patients living with VWD.

Overview of VWD

VWD is characterized by three main disease subsets with varying degrees of quantitative and qualitative defects. In most cases, VWD is inherited; however, milder forms of inherited VWD may not be symptomatic in some individuals and in rare cases, it can be caused by a genetic mutation with no prior family history of the disease. There are also a handful of disorders that may cause acquired, non-inherited forms of VWD.

Type 1 VWD is a quantitative issue in which a low level of VWF is present in a patient's blood, with a potential for accompanying low FVIII levels. Type 1 VWD is mildly symptomatic, comprising 70-80% of all VWD diagnoses. Reduced VWF levels result in decreased ability of platelets to adhere to sites of vascular injury. Type 1C is a rare form of Type 1 VWD and is identified by increased clearance of matured Von Willebrand factor molecules, leading to low VWF levels. Type 1 VWD symptoms include mild mucosal bleeding and seldom more serious symptoms.

Type 2 VWD is characterized by qualitative defects in the VWF protein. With varying degrees and types of protein dysfunction, all subsets of Type 2 VWD are linked by the fact that the protein lacks a critical function, and the patient will require treatment of bleeding symptoms.

- In **Type 2A**, VWF polymers (known as multimers) are shorter than normal. The shortened VWF fails to bind to platelets, reducing the body's ability to create a platelet plug at the site of vascular injury.
- In **Type 2B**, the patient's VWF is too easily activated to bind platelets. As a consequence, platelets may clump together with VWF in the blood stream, resulting in clearance of both VWF and platelets from circulation. Patients with Type 2B have an increased risk of developing thrombocytopenia.
- **Type 2M** is characterized by decreased VWF and its failure to interact with platelets, leading to moderate mucosal bleeding with the potential of developing severe bleeds.
- Patients with **Type 2N** experience a failure of VWF to transport FVIII to an injury site and have reduced

levels of FVIII in their blood. Type 2N often resembles hemophilia A and patients with this subset can develop severe bleeding following surgery if not managed properly.

Type 3 VWD is the most severe and is extremely rare, occurring in approximately one in every one million people. In Type 3, patients experience a near complete or total absence of VWF in the blood. Symptoms include severe mucosal bleeding, joint bleeds, joint damage and hematomas. Women can experience life-threatening symptoms with reproductive tract bleeding and postpartum hemorrhage.

Acquired VWD usually occurs as a consequence of another medical condition, such as lymphoproliferative disease, autoimmune disease, or valvular heart disease. Patient symptoms and their Von Willebrand lab parameters may mimic type 1, type 2A or type 3 VWD.

Challenges of Diagnosis

> "Confidence in diagnosis more efficiently guides treatment decisions. When treatments are guided more precisely, quality of life and patient outcomes are improved." – Kenneth Friedman, MD, Senior Medical Director, Hematology (Mann Jackson, 2017)

There is no "one-size-fits-all" approach for accurate VWD diagnosis. It presents numerous challenges due to the variations in disease subsets, inconsistencies with testing protocols throughout the medical community, and variability in VWF reference ranges for disease types. Precise diagnosis of VWD involves a combination of characteristic symptoms, patient and family histories, clinical evaluation, and specialty testing.

In 2020, the American Society of Hematology (ASH), along with the International Society on Thrombosis and Haemostasis (ISTH), National Hemophilia Foundation (NHF) and World Federation of Hemophilia (WFH), established a multidisciplinary guideline panel to develop and publish diagnostic recommendations to reduce the variability of VWD diagnosis around the world. The panel agreed on 11 recommendations, including guidelines for the use of bleeding assessment tools (BATs) in patients suspected of VWD, diagnostic assay and laboratory cutoffs for Type 1 and Type 2 VWD, how to approach a Type 1 VWD patient whose VWF levels normalize over time, and the role of genetic testing versus phenotypic assays for types 2B and 2N. (James et al., 2021)

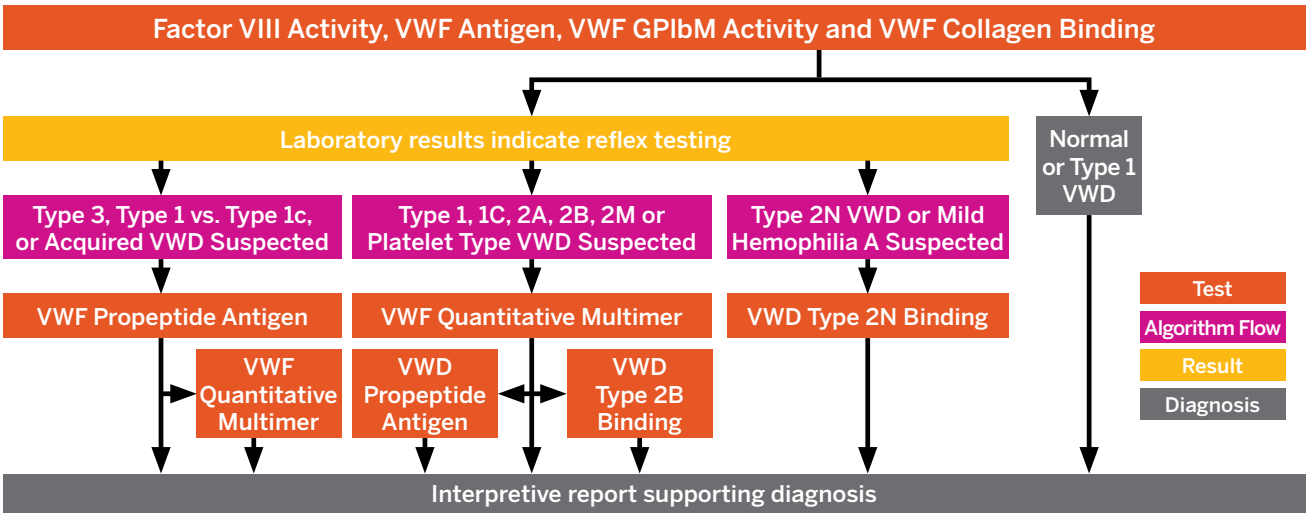
When initiating an evaluation for suspected VWD, specific assays are used to measure the activity of various proteins in the blood. Tests include the VWF antigen test to measure VWF levels in the blood; FVIII clotting activity tests to measure how well the patient's FVIII is working; and other assays, such as the ristocetin cofactor assay (VWF:RCo) or GP1bM assay (VWF:GP1bM), to measure platelet-binding activity. Results from these assays will indicate if additional testing is required to determine the subtype of VWD present. These tests can include the VWF multimer test to examine the length of VWF polymers, tests that evaluate platelet collagen, or factor VIII binding function, or genetic testing to determine the gene mutation causing the disorder.

VWD can be both under- and over-diagnosed due to its lack of consistency among laboratories around VWF reference ranges for Type 1 diagnosis. The differentiation between true Type 1 and "low VWF levels" is challenging. Further adding to the complexity of diagnosis, people with blood group type O naturally have low VWF levels and can be asymptomatic, whereas people with A, B or AB type blood can have VWF levels similar to those with type O blood yet experience VWD symptoms.

Another issue contributing to the misdiagnosis of VWD is utilization of the ristocetin cofactor assay VWF:RCo, which uses an antibiotic to induce platelet binding. VWF:RCo has several limitations, including the fact that it can be affected by a common benign VWF sequence variation—specifically, an A1 domain single nucleotide polymorphism (D1472H) affects VWF binding to ristocetin without altering in vivo activity. This polymorphism has no clinical significance but can lead to a laboratory misdiagnosis of VWD. (Flood et al., 2010) The multidisciplinary guidance panel recommends utilizing assays that measure the VWF-platelet binding activity that are not adversely affected by the D1472H polymorphism, such as VWF:GP1bM or VWF:GP1bR, over VWF:RCo. (James et al., 2021)

Proper VWD diagnosis is critical for providing essential treatment. Often, physicians do not consistently perform the same battery of tests on all patients, creating potential gaps in diagnostic information. Relying upon the results of only one assay or a limited VWD testing panel can lead to misdiagnosis.

A Comprehensive Testing Solution



To alleviate the guesswork among multiple assays, Versiti has developed a single orderable that provides actionable diagnostic information. Versiti's Von Willebrand Disease Diagnostic Evaluation is a reflexive testing algorithm used to detect quantitative or qualitative defects of VWF and related deficiency of FVIII activity, differentiate subtypes of variant VWD, and direct optimal utilization of confirmatory genetic testing for variant VWD.

Versiti's VWD diagnostic reflexive algorithm was developed by our clinical and technical experts, incorporating our expertise along with peer-reviewed diagnostic and clinical guidelines, such as those from ASH and the National Heart, Lung and Blood Institute (NHLBI). The diagnostic evaluation begins by concurrently performing the FVIII activity, VWF antigen, VWF GP1bM activity and VWF collagen III binding assays. Then, information gathered from the first battery of assays is used to determine which, if any, additional assays should be reflexively performed. For example, a low collagen III binding to VWF antigen ratio indicates abnormal multimers, prompting confirmatory testing through use of the VWF quantitative multimer assay.

Where acquired VWD or Type 3 is suspected, or distinction of Type 1 versus Type 1C (VWF antigen <30 IU/dL) is needed, the VWF propeptide antigen assay will be performed. Based upon the results from the propeptide antigen assay, further confirmatory testing may be done using Versiti's VWF quantitative multimer assay to analyze the size distribution of VWF polymers. In cases where Type 1, 1C, 2A, 2B, 2M, or platelet-type Von Willebrand disease is suspected (VWF antigen <30 IU/dL or abnormal ratio of VWF:CBIII/VWF:Ag ratio), the VWF quantitative multimer assay is performed first. Then, reflexive confirmatory testing is performed as needed, such as the VWD Type 2B binding assay. In some patients with Type 1C, multimer analysis may reveal the presence of ultra-large-molecular-weight multimers, likely explained by high turnover of VWF that does not give enough time for ADAMTS13 to cleave to VWF, resulting in the presence of recently secreted, ultra-large-molecular-weight multimers in plasma. (Ng, et al., 2015)

If Type 2N VWD or mild hemophilia A is suspected from the initial set of assay results, the VWD Type 2N binding assay will be run to determine if a defect of VWF is responsible for low factor VIII in the patient. If additional information related to treatment or long-term patient management is needed following completion of the diagnostic evaluation, Versiti will recommend confirmatory genetic testing. Finally, there are rare cases of Type 2M VWD attributable to abnormal interaction of VWF with collagen IV. If the VWD Diagnostic Evaluation shows normal VWF levels and function, and the patient has a significant history of bleeding, Versiti's collagen IV binding assay (order code: 1280) could be added by the ordering physician. Because this additional testing requires clinical input, it is not part of the reflexive panel and would need to be ordered by the patient's physician. The Versiti lab holds samples for 2 months to allow the referring physician to add on clinically indicated testing.

"As the bleeding pattern can affect quality of life and even survival, it is essential to optimize the diagnostic process in order to ensure the most adequate therapeutic strategy promptly in patients presenting with spontaneous bleeding."
(Schinco et al., 2018)

Timing is critical for patient outcomes in the diagnosis of bleeding disorders. Because testing is done on a reflexive basis, Versiti's VWD Diagnostic Evaluation has a standard turnaround time of 14 days, eliminating days or weeks that may be wasted by ordering tests and waiting for results from multiple individual assays. For example, if a physician followed Versiti's Von Willebrand diagnostic algorithm but placed orders for standalone tests in succession, turnaround time for results could exceed 21 days for a case of Type 2 VWD. By requesting a single orderable, overall time to diagnosis is drastically reduced and accuracy in diagnosis is improved. Following completion of the VWD Diagnostic Evaluation, Versiti delivers physicians an interpretive report, providing actionable diagnostic insight, including recommendations for effective disease management.

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Conclusion

Von Willebrand disease is a common, yet complex, set of bleeding disorders. Executing a piecemeal approach to VWD testing leads to inconsistent results, hindering timely diagnosis and disease management. The Versiti VWD Diagnostic Evaluation was created to eliminate the variability in testing protocols and provide physicians and their patients with actionable diagnostic information. Versiti is proud to offer the most robust and comprehensive VWD testing portfolio available in the United States.

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For more information about or to order the Versiti Von Willebrand Disease Diagnostic Evaluation, visit versiti.org/VWDevaluation

Sources

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