Laboratory Evaluation of von Willebrand Disease

von Willebrand disease (vWD) is the most common inherited bleeding disorder. Laboratory studies have shown a prevalence of 1–3% in several populations, although the incidence of clinical disease is lower (1–3). vWD is manifest by easy bruising and prolonged bleeding, especially from mucosal surfaces. Most patients have a quantitative deficiency of von Willebrand factor (vWF), but ~20% produce a qualitatively abnormal protein. vWF is found in the subendothelial matrix, as well as in plasma and in the platelet α-granule, from which it is released upon platelet aggregation (4, 5). vWF mediates initial platelet adhesion to the blood vessel wall at the site of injury. Interaction of vWF with the platelet GpIb–IX–V receptor results in platelet activation through poorly understood signaling pathways that mediate exposure of functional platelet GpIb–IIIa (α₉β₃) receptors and subsequent platelet aggregation (reviewed in ref. 6). vWF also plays a role in mediating platelet–platelet interaction via the GpIb–IIIa receptor. Factor VIII, the coagulation factor deficient in hemophilia A, binds to and is stabilized by vWF. Thus, the concentration of factor VIII usually correlates with that of vWF protein.

vWF is synthesized by endothelial cells and megakaryocytes. This process has been studied extensively in endothelial cells (reviewed in ref. 7), where vWF is synthesized as propolypeptides that form disulfide-linked dimers through C-termini shortly after synthesis in the endoplasmic reticulum. During transit through the Golgi apparatus, the dimers multimerize by disulfide bonds at N-termini, and the propeptide sequence is cleaved. vWF is either secreted constitutively or stored within the Weibel–Palade body, from which it is secreted in response to several agonists. vWF multimers range from 0.6 to 20 × 10⁶ Da. Multimerization is critical in producing functional vWF: Only the larger multimers appear hemostatically active.

Diagnosis of vWD depends on the use of several laboratory tests along with clinical assessment. In the past, >20 distinct clinical and laboratory subtypes were described, making classification difficult for all but the most experienced laboratories. A new classification of vWD recently proposed is simpler, in that there are fewer types, and more clearly distinguishes quantitative from qualitative defects (8). In this classification, type 1, accounting for ~80% of vWD, is due to a partial quantitative deficiency of vWF. Type 2 vWD refers to qualitative abnormalities of vWF; of these, type 2A, which represents decreased function associated with loss of high-M₇ multimers, is the most common. Type 2B refers to qualitative variants with increased affinity to the platelet GpIb receptor; it is also associated with loss of the highest-M₇ multimers, although less so than type 2A, and with thrombocytopenia because of abnormal binding in vivo of vWF to platelets, with subsequent clearance from the circulation. Type 2M refers to functional defects without loss of high-M₇ multimers, and type 2N to vWD with abnormal factor VIII binding to vWF, sometimes referred to as autosomal hemophilia. Type 3 refers to severe vWD with near or complete absence of vWF antigen.

Laboratory analysis for vWD includes determination of vWF quantity, structure, and function. vWF antigen quantities are determined most commonly by quantitative immunoelectrophoresis or ELISA. Determination of blood-type is important in the initial evaluation of vWD because the amounts of antigen vary significantly with blood type (9). People with type AB blood have the highest concentrations of vWF antigen, which can be almost twice that of people with type O, who have the lowest levels. The reason for this difference is unknown, but the reality is that vWD may be under- or overdiagnosed if vWF antigen concentrations are interpreted without knowledge of the patient’s blood type. Multimer analysis shows the vWF structure, and loss of high-M₇ multimers suggests a qualitative abnormality; however, functional assays are needed because qualitative abnormalities may exist with a normal multimer distribution. Additionally, multimer analysis is a costly and labor-intensive test and is usually done only with the initial patient evaluation.

The bleeding time, which classically is prolonged in vWD, is neither very sensitive nor specific. It may be normal in some patients with vWD, and prolonged in several conditions other than vWD, including drug ingestion and intrinsic platelet disorders. The activated partial thromboplastin time (aPTT) is prolonged in vWD if the factor VIII concentration is decreased sufficiently but will be normal in many cases of vWD.

The observation by Howard and Firkin in 1971 (10) that ristocetin induces platelet agglutination in normal platelet-rich plasma but not in plasma from a patient with severe vWD has become the basis for the laboratory measurement of vWF function. Ristocetin allows vWF to bind to the platelet GpIb receptor in solution. The ristocetin–vWF interaction is affected by charge and by glycosylation of vWF (11, 12). Classic ristocetin-induced platelet aggregation is not very sensitive to quantitative or qualitative abnormalities of vWF. Its use in the characterization of vWD is to detect an increased response to low concentrations of ristocetin in type 2B vWD.

The ristocetin cofactor assay, addressed by Ermens et al. in this issue (13), was developed originally by Weiss et al. (14) and is now the most widely used test
to assess vWF function. In the assay, dilutions of patient plasma are mixed with standard concentrations of normal platelets and ristocetin, and agglutination is monitored by platelet aggregometry or visual inspection. This test is more sensitive for diagnosis of type 1 vWD than is measurement of the vWF antigen concentration (with cutoffs for both tests placed at the upper 90% confidence interval of the 2.5 percentile of a reference population) (15). The ristocetin cofactor assay also had significantly higher diagnostic accuracy (by receiver-operator characteristic curve analysis) than vWF antigen (P < 0.05) or factor VIIIc (P < 0.01) in a study of 24 children presenting with a history of mucosal bleeding (16). Furthermore, the possibility of a significant disparity between antigen quantity and function, as in type 2 disease, the ristocetin cofactor assay, along with the factor VIII concentration, is probably the most critical test for guiding appropriate therapy and response.

The mainstay in treatment of mild to moderate vWD is the synthetic vasopressin analog desmopressin (1-desamino-8-D-arginine vasopressin; DDAVP), which induces release of stored vWF. Somewhat surprisingly, many patients with type 2A vWD respond to this therapy with sufficient improvement in the bleeding time and ristocetin cofactor activity to avoid treatment with plasma-derived products. Additionally, the current treatment of choice in patients who do not respond sufficiently to DDAVP, or whose clinical situation requires therapy other than DDAVP, is to use virally inactivated factor VIII products that retain some vWF activity. Because the larger-M₈ multimers may be lost in the manufacturing process, the ristocetin cofactor activity in these products and the patient’s plasma activity in response to these therapies are important markers of efficacy.

The laboratory diagnosis of vWD remains problematic, in that all values may be within reference (normal) limits in patients with vWD, and the values may vary over time, possibly due to the effects of hormones and acute-phase reactants on vWF biosynthesis. Because vWD is inherited as an autosomal dominant trait, family studies are helpful—although values and symptoms frequently vary among family members. Normal values in patients with vWD do not preclude an increased risk of bleeding. Advances in molecular diagnostics have elucidated some of the mutations resulting in vWD, especially in patients with type 2 disease (reviewed in ref. 17). However, this information can only in the broadest sense predict functional quantities of vWF; hence, the ristocetin cofactor assay remains our best test for vWF function. The use of standard lyophilized fixed platelets may help improve the reliability of this test, but, as Ermens et al. (13) point out, results may vary widely, depending on platelet source. The ristocetin cofactor assay performed with the commercially available slide test, although still requiring technical expertise, requires less time and equipment does platelet aggregometry. Ermens et al. found that the slide test has less interassay variability than either the commercially available assays utilizing aggregometry or the classic test described by Weiss et al. (14). Interassay variability also was less than that reported by Puri et al. (18), who used an ELISA plate reader to detect changes in light absorbance with platelet agglutination. As in all studies of this nature, the contribution of an experienced technologist, especially in an assay requiring observation (such as for platelet agglutination), must be remembered, and the variability in many clinical laboratories is likely to be greater. However, until we have a better test for vWF function, improvements in the ristocetin cofactor assay should help us more quickly and accurately diagnose and measure response to therapy in vWD.

References

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