Elderly Female with a Personal and Family History of a Bleeding Disorder

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CASE DESCRIPTION

A 77-year-old woman with a history of excessive bleeding and questionable diagnosis of von Willebrand disease (VWD) presented for a presurgical evaluation. Her daughter and grandson also had a history of a bleeding disorder. Bleeding events reported by the patient and her family members included easy bruising, postsurgical bleeding and hematoma formation requiring intervention with drainage and blood products, and excessive bleeding during childbirth. The physician requested testing for VWD to confirm the diagnosis and subtype, since subclassification of VWD guides appropriate therapy. Results of the initial testing revealed typical prothrombin time (PT) (13.7 s, reference interval 12.0–15.5 s), typical activated partial thromboplastin time (aPTT) (31.5 s, reference interval 24–35 s), mild thrombocytopenia (116 K/µL, reference interval 150–450 K/µL), typical von Willebrand factor antigen level (VWF:Ag) (108%, reference interval 52%–214%), typical factor VIII activity (98%, reference interval 56%–191%), decreased von Willebrand factor ristocetin cofactor activity (VWF:RCo) (19%, reference interval 51%–215%), and decreased VWF:RCo/VWF:Ag ratio (0.18, reference interval 0.7–1.0).

DISCUSSION

Inherited bleeding disorders arise from abnormalities in primary hemostasis (formation of the initial platelet plug), secondary hemostasis (fibrin clot formation), or rarely from fibrinolytic or combined disorders. Initial evaluation of a suspected bleeding disorder includes a thorough personal and family bleeding history, complete blood count (CBC) with platelet count and peripheral smear review, PT, and aPTT. Fibrinogen measurement is also useful, since the PT and aPTT tests are not always sensitive to clinically significant fibrinogen disorders. Fig. 1 summarizes typical test result patterns in inherited bleeding disorders. If the patient has a strong personal or family history of mucocutaneous bleeding, VWD testing should be pursued early in the workup. The patient in this case had typical PT and aPTT with mild thrombocytopenia and a mucocutaneous bleeding history, which led to a workup for VWD.

Current guidelines for initial evaluation of VWD recommend the following tests: (a) VWF:Ag, (b) VWF:RCo, and (c) factor VIII (1). VWF:Ag quantifies total VWF; VWF:RCo is a functional test in which VWF agglutinates platelets in the presence of ristocetin, which “activates” VWF, allowing it to interact with platelet GP1b (2). Diagnosis of the most common form of VWD (type 1) can usually be made with results from this initial testing, although additional testing may be necessary for diagnosis and subclassification of the less common subtypes. VWF multimeric analysis by gel electrophoresis is a useful test for subclassification but is not indicated in the initial workup of inherited VWD. In this case, electrophoresis revealed absent high molecular weight multimers (Fig. 2).

VWF is a multimeric glycoprotein, comprising high, intermediate, and low molecular weight (HMW, IMW, and LMW) multimers (1). VWF functions to stabilize factor VIII and support platelet hemostasis. VWF binds subendothelial collagen and adheres to platelets via the platelet GP1b receptor, which allows platelet clots to form at sites of injury. The HMW multimers are most effective at promoting platelet hemostasis (2). Common symptoms of VWD include mucocutaneous
bleeding such as easy bruising, frequent or prolonged nose bleeds, menorrhagia, and prolonged bleeding after trauma, childbirth, or surgery (1).

VWD subtypes have different laboratory patterns, bleeding phenotypes, and treatment approaches (1). Types 1 (autosomal dominant, 70%–80% of cases) and 3 (autosomal recessive, <5% of cases) are quantitative deficiencies due to either decreased (type 1) or absent (type 3) VWF, with a typical multimeric distribution (if protein is present). The type 2 subtypes (autosomal dominant or recessive, 10%–15% of cases) involve functional defects in VWF. Atypical VWF function can be a result of missing VWF multimers (type 2A, type 2B, and platelet-type) or loss-of-function mutations in the platelet binding domain (type 2M) or factor VIII binding domain (type 2N) of VWF. VWF multimeric analysis is used for subclassification when a type 2 disorder is suspected.

If VWF:RCo is decreased in proportion to VWF:Ag (concordant, reference VWF:RCo/VWF:Ag ratio 0.7–1.0), a quantitative type of VWD (type 1) should be suspected (1). If there is a disproportionate decrease between VWF activity and antigen (discordant, VWF:RCo/VWF:Ag <0.7), then type 2A (autosomal dominant or recessive), 2B (autosomal dominant), 2M (autosomal dominant), or platelet-type VWD (PT-VWD, autosomal dominant) should be considered. In types 2A, 2B, and PT-VWD, the ability of VWF to agglutinate platelets in the presence of ristocetin is disproportionately decreased owing to the absence of HMW and IMW (2A) or HMW (2B, PT-VWD) multimers, resulting in a markedly decreased VWF:RCo. In type 2M, there is a defect in the platelet-binding domain of VWF resulting in decreased VWF:RCo, but all multim-

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<th>Fig. 1. Typical platelet count, PT, and aPTT patterns in inherited bleeding disorders.</th>
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<td>Although VWD commonly presents with platelet count, PT, and aPTT within reference intervals, some forms of VWD show thrombocytopenia and/or prolonged aPTT. Some forms of inherited platelet dysfunction show associated thrombocytopenia. Depending on the pattern observed, additional workup could include VWD testing panel, platelet function studies, fibrinogen evaluation, factor assays, assays of fibrinolytic components, or peripheral blood or bone marrow evaluation. N, normal; ↑, prolonged; ↓, decreased.</td>
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<th>Fig. 2. Von Willebrand multimeric analysis by gel electrophoresis.</th>
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<td>The patient sample (lane 8) demonstrated an absence of HMW multimers. Lanes 1, 6, and 10, typical control; lane 2, atypical control (HMW multimers absent); lane 8, patient sample.</td>
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ers are present (1). VWF:Ag, which tests for the total amount of VWF, may be relatively normal or only slightly decreased in these subtypes, since only the larger multimers are missing (2A, 2B, PT-VWD) or all multimers are present (2M).

Factor VIII measurement is important for initial testing, because VWF carries factor VIII. In addition, in the rare 2N subtype (autosomal recessive), there is a mutation in the factor VIII binding domain of VWF, which results in decreased factor VIII (1). Type 2N may be misdiagnosed as hemophilia A, but if the affected individuals are of both sexes, hemophilia A is unlikely because it demonstrates an X-linked inheritance pattern. Additional testing such as VWF:factor VIII binding activity or genetic testing may be necessary to differentiate these 2 disorders.

This patient’s laboratory data revealed a decreased VWF:RCO/VWF:Ag ratio, absent HMW multimers, and mild thrombocytopenia, suggesting a qualitative subtype of VWD with missing multimers, thus type 2B, PT-VWD, or possibly type 2A. Low-dose ristocetin-induced platelet aggregation studies (LD-RIPA) were subsequently performed and demonstrated increased aggregation, indicating a high affinity between the patient’s platelets and VWF, narrowing the differential diagnosis to either type 2B or PT-VWD. The defect in type 2B VWD (5%–8% of all VWD cases) is a gain-of-function mutation in VWF that causes an increased affinity for the platelet GP1b receptor, resulting in continuous formation and clearance of VWF-platelet complexes (1–3). Clearance of these complexes results in loss of the HMW multimers and mild thrombocytopenia. In contrast, PT-VWD (exact prevalence unknown, very rare, many cases are misdiagnosed as type 2B) involves a high-affinity mutation in the platelet GP1b receptor, which causes increased binding to VWF (4). Thus, the clinical presentation (mild or moderate bleeding) and laboratory profile for these disorders are similar.

The traditional LD-RIPA test is performed on platelet-rich plasma, which contains patient platelets and VWF and does not allow differentiation of type 2B and PT-VWD. Traditional options for differentiation include VWF:platelet binding assays or specialized LD-RIPA aggregation mixing studies (1), which help to determine whether the defect lies in the patient’s VWF or their platelets. However, these specialized tests are not widely available and may not allow for definitive diagnosis. In cases such as this, genetic testing is a useful tool for identifying the pathogenic mutations.

Genetic testing was performed in this case to differentiate type 2B and PT-VWD. Eighty percent of VWF mutations causing type 2A, 2B, and 2M are located in exon 28 of VWF(5) (von Willebrand factor) (5). PCR analysis followed by bidirectional sequencing of exon 28 can detect type 2B mutations. Because type 2B VWD is more common than PT-VWD, testing for type 2B mutations should be performed first. If no mutations are detected, testing should be considered for GP1BA [glycoprotein Ib (platelet), alpha polypeptide] mutations to look for PT-VWD (1, 3). This involves PCR followed by targeted mutation analysis of the 4 previously identified mutations: c.746G>T (p.Gly249Val), c.746G>A (p.Gly249Ser), c.763A>G (p.Met255Val), and c.1306del27 (p.436_444del9) (1, 6).

Treatment for type 2B VWD and PT-VWD differs dramatically (1). 1-Deamino-8-D-arginine vasopressin is contraindicated in type 2B, as more defective VWF would be released into the circulation, causing greater clearance of large multimers and platelets. VWF concentrates are used to treat type 2B, and treatment for PT-VWD involves transfusion of platelets.

**CASE FOLLOW-UP**

VWF gene sequencing of exon 28 was performed first and no mutation was detected. GP1BA targeted mutation testing was subsequently performed and resulted in detection of 1 of 4 known PT-VWD mutations (c.763A>G, p.Met255Val, according to standard nomenclature). This confirmed the diagnosis and guided

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**POINTS TO REMEMBER**

- VWD is broadly subclassified into quantitative (types 1 and 3) or qualitative (types 2 and PT-VWD) subtypes by use of tests to evaluate protein amount, activity, factor VIII activity, and multimeric distribution.
- Type 2B and PT-VWD both involve a decreased VWF:RCO/VWF:Ag ratio, absence of HMW VWF multimers, and mild thrombocytopenia. These types are indistinguishable by routine laboratory testing. Testing to distinguish these 2 subtypes is not widely available.
- The defect in type 2B VWD involves a gain-of-function mutation in VWF; in PT-VWD, there is a gain-of-function mutation in the GP1b platelet receptor.
- Therapies differ dramatically depending on VWD subtype. Treatment for type 2B VWD involves administration of a VWF concentrate to supplement VWF. Treatment for PT-VWD is the transfusion of platelets.
- Gene sequencing analysis is effective in helping to confirm and differentiate subtypes of type 2 VWD, including type 2B and PT-VWD, which is an essential distinction in guiding appropriate therapy.
appropriate therapy. It also provided a definitive diagnosis for the patient’s affected family members in this autosomal dominant disorder.

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**References**


**Commentary**

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Von Willebrand factor (VWF)2 is a large and complex protein with many diverse hemostatic functions, of which binding to (a) platelet glycoprotein Ib (GPIb), (b) subendothelial collagen, and (c) FVIII are best known. Deficiencies or defects in VWF give rise to von Willebrand disease (VWD), the most common inherited bleeding disorder. A correct diagnosis is critical and relates to management; rather than one size fits all, different therapies are applied to different VWD types. VWD is classified into 6 types, with quantitative deficiencies assigned to types 1 (partial deficiency) and 3 (total deficiency). Qualitative defects are classified into type 2, with 2A VWD defined by loss of high molecular weight (HMW; largest forms of) VWF, type 2B reflecting hyperadhesive VWF [but phenotypically also presenting loss of HMW VWF, often with (mild) thrombocytopenia], type 2N defined by defective binding to FVIII, and type 2M representing a variety of functional defects. Treatment of VWD is most typically achieved by replacement VWF concentrate, occasionally supplemented by other therapies. Platelet-type VWD (PT-VWD) is not classified within the standard VWD scheme, as it represents a defect in the GPIb molecule. Phenotypically, however, PT-VWD is often indistinguishable from 2B VWD because of the similar loss of HMW VWF and mild thrombocytopenia, albeit via a different mechanism. PT-VWD can be diagnosed only by specialized testing (ristocetin-induced platelet aggregation mixing studies) and genetic testing of GPIBA (glycoprotein Ib (platelet), alpha polypeptide). Given that defects lie in GPIb, replacement VWF is inappropriate therapy since it will just lead to increased clearance of the hyperadhesive platelets. Thus, management of PT-VWD requires platelet replacement. Although PT-VWD is considered a rarer form of VWD than 2B VWD, with a presumed prevalence at approximately 10% that of 2B VWD, the fact that PT-VWD is so commonly misdiagnosed as other disorders, or otherwise remains undiagnosed after standard investigations, underinforms on the actual prevalence.

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