Von Willebrand Disease: Range of the Disease, and Management

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Abstract Von Willebrand disease is the most common autosomal inherited bleeding disorder. It is caused by quantitative or qualitative defects of the von Willebrand factor. The International Society of Thrombosis and Hemostasis recognizes three types of Von Willebrand disease, with four qualitative subtypes, i.e. six different groups in total. All variants present with mucocutaneous bleeding of variable severity depending on the penetrance of the disease, the level of von Willebrand factor (VWF), and the specific abnormality of the defect, resulting in altered VWF interactions between either platelets and collagen or factor VIII. Diagnosis is difficult because the clinical and laboratory phenotypes are very heterogeneous and may overlap for normal subjects. The molecular pathology of the condition corresponds to the specific variants but has a wide range of genetic mechanisms. Accurate diagnosis of the disorder is of critical importance to establish appropriate treatment options for individual patients. This review covers the pathophysiology and genetics of the condition, the diagnostic classification, testing, and the available treatments, specifically highlighting the population.

Keywords Von Willebrand disease (VWD) · Children · Management

Introduction

Von Willebrand disease (VWD), the most common inherited bleeding disorder, results from deficiency or functional abnormalities of the von Willebrand factor (VWF). It is an autosomal disorder with variable penetrance. Although the prevalence of laboratory diagnosis of VWD among the general population is estimated to be approximately 1 %, recent reports have indicated that the prevalence of symptomatic VWD in unselected patients presenting to family practice offices is 0.09 % [1, 2].

Von Willebrand Factor

The VWF protein is encoded by a gene on the short arm of chromosome 12 and synthesized in the endothelial cells and megakaryocytes. Chromosome 22 also carries a partial VWF pseudo gene that replicates VWF gene sequences in chromosome 12 between exon 23 and 34 with 3 % variance [3]. The protein undergoes complex post-translational modifications, including dimerization, glycosylation, sulfation, and multimerization. Once fully processed, the protein is released into the circulation or stored in the Weibel–Palade bodies of endothelial cells or the α -granules of platelets. In the plasma, VWF is a large multimeric protein with a molecular weight that ranges from 500 to 2,000 kDa. The high-molecular-weight VWF multimers undergo partial proteolysis mediated by the ADAMTS-13 protease [4].

The multimeric nature of VWF protein renders VWF capable of binding both platelets and collagen. Its function

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is to localize platelets to the site of bleeding by binding to collagen and also to platelets that are traveling through the injured endothelium. The binding of platelets to collagen by VWF leads to platelet aggregation and the formation of a platelet plug, the very initial component of the hemostatic process that ultimately leads to cessation of blood flow. VWF serves as a carrier protein for the procoagulant factor VIII. Factor VIII (FVIII) circulates in the bloodstream in an inactive form, bound to VWF until an injury that damages blood vessels occurs. In response to injury, FVIII separates from VWF and becomes activated. Activated FVIII interacts with coagulation factor IX. This interaction sets off a chain of additional chemical reactions that ultimately form a blood clot.

VWF serves two important roles in primary and secondary hemostasis:

- (1) Mediator of platelet adhesion to injured endothelium through its interaction with GP1b/IX receptor on platelets, collagen in the damaged endothelium, and platelet aggregation via platelet to platelet interaction between GPIIb/IIIa integrin after platelet activation [5, 6].
- (2) Carrier and stabilizing protein for the procoagulant factor VIII. FVIII circulates in the bloodstream in an inactive form while bound to VWF, until damage to blood vessels occurs. In response to injury, FVIII separates from von VWF and becomes activated,

which then interacts with factor IX and sets off a chain of additional chemical reactions that ultimately form a blood clot [7].

VWD Classification and Genetics

VWD is the result of a quantitative or qualitative disorder. The International Society on Thrombosis and Hemostasis (ISTH) established three types of VWD: type 1 VWD, a defect that results in a reduced amount of normally functioning VWF (hypoproteinemia); type 2 VWD, a qualitative deficiency of VWF that results in functionally abnormal VWF (dysproteinemia), and type 3 VWD, in which the VWF protein is completely absent (aproteinemia) [8]. Clinical and genetic aspects of VWD are summarized in Table 1.

Type 1 VWD

Type 1 VWD is a mild to moderate quantitative deficiency (15–50 % level) of VWF with normal multimer structure. It is the most common form of VWD, accounting for 80 % of the cases, and it is inherited as an autosomal dominant disorder. Sixty-five percent of patients with type 1 VWD have mutations in the VWF gene, most commonly a missense mutation that ultimately results in a quantitative reduction of qualitatively normal VWF protein. Some

Table 1 Classification and genetics of VWD

VWD type	Prevalence	Inheritance	Mutation location	Pathophysiology	Clinical and laboratory findings
Quantitative deficiency					
1	1 % of general population 80 % of VWD	Autosomal dominant	Several mutations resulting in null alleles ~ 15 % >1 mutation	Reduced amount of normal VWF Accelerated clearance	Low risk of bleeding complications
3	1 in 250,000 people 1–5 % of VWD	Autosomal recessive	Spread over entire VWF gene	Complete absence of VWF	Risk of hemophilia-related bleeding (i.e. joint hemorrhages) Severe menstrual bleeding at menarche
Qualitative deficiency					
2A	10 % of VWD	Autosomal dominant	A2 domain 80 % C2 10 % D2 10 % D3	Loss of HMW and intermediate VWF multimers	
2B	Rare	Autosomal dominant	A1 domain	Gain-of-function of VWF to binding to GP1b platelet receptor	Thrombocytopenia
2M	Rare	Autosomal dominant	A1 domain GP1ba A3 domain collagen	Loss-of-function of VWF to binding to GP1b platelet receptor Normal multimer structure	
2N	Rare	Autosomal recessive	D'D3 domain	Abnormal VWF binding site for FVIII	Resembles hemophilia A

patients have no obvious mutations, but >15 % may have more than one mutation [4•, 9••]. Some patients may have only mild symptoms and mildly reduced VWF levels, whereas others are more severely affected because of the variable penetrance of the disorder.

Type 2 VWD

Type 2 VWD is a qualitative defect in VWF activity which is further subdivided into four subtypes. In all four subtypes, a mutant VWF protein with abnormal function is produced. Types 2A, 2B, and 2M have qualitative defects that affect the VWF–platelet interactions; type 2N has defects in interaction of VWF with FVIII.

Most of the genetic mutations in type 2 VWD are the result of missense mutations affecting the region of the VWF protein involved in binding to platelets, collagen, or FVIII. Types 2A, 2B and 2M have a dominant inheritance pattern with complete penetrance of the phenotype in heterozygotes. Type 2N is transmitted as a recessive trait requiring the inheritance of either homozygous or compound heterozygous 2N mutations (either two different type 2N missense mutations or a combination of a 2N missense mutation with a VWF null mutation) to manifest the low levels of FVIII [10].

Type 2A VWD is the most common of the type 2 variants. It is characterized by the loss of high and intermediate-molecular-weight multimers. It is a loss of platelet-dependent function phenotype because of the presence of abnormal multimers that are essential to platelet adhesion to exposed sub endothelial matrix. The characteristic of type 2A disease is a low VWF:RCO-to-VWF:Ag ratio of <0.6 and impaired ristocetin induced platelet agglutination (RIPA).

More than 70 mutations have been described for this phenotype, with more than 80 % of them located in exon 28. The defect results in two different pathogenic mechanisms, one that results in defective synthesis of high-molecular-weight multimers and the other in an enhanced propensity for ADMATS-13 mediated proteolysis. Others have suggested genetic mutations that result in complex abnormalities including synthesis, storage, release, and proteolytic processing [11].

Type 2B VWD is a consequence of genetic mutations that result in increased binding of VWF to platelets, or a “gain-of-function” phenotype in the GPIIb-binding site on VWF that leads to increased interaction with platelets. The mutation results in a change of conformation of the A1 domain from a resting state to a hyperactive configuration. Laboratory testing that suggests this variant includes VWF:RCO-to-VWF:Ag ratio <0.6, depletion of high-molecular-weight multimers, enhanced RIPA, and thrombocytopenia. More

than 20 different mutations involving exon 28 affecting residues at the base of 2A1 domain that interact with GPIIb have been described [12].

Type 2M VWD is characterized by mutations in the GPIIb binding domain of VWF that result in “loss-of-function” with failure of the mutant VWF to bind normally to GPIIb. In type 2M, high-molecular-weight multimers have normal structure, which differentiates the disorder from type 2A VWD. This type is associated with a milder bleeding phenotype than the other VWD [13]. Some dysfunctional variants of 2M type have abnormal binding to collagen, particularly collagen types I, III, and VI, and therefore have abnormal VWF collagen binding activity (VWF:CB). These variants are likely to have missense mutations in the A3 domain, instead of the most common mutation in the A1 domain. Laboratory evaluation of these patients reveals low VWF:RCO-to-VWF:Ag ratio, normal multimer structure and reduced RIPA. Some 2M cases may be difficult to differentiate from type 1 VWD; making the distinction between the two types is, however, of therapeutic importance, because administration of desmopressin will benefit type 1 patients only and will not rescue the loss-of-function characteristic of type 2M patients.

Type 2N VWD is the result of a mutation in the FVIII binding site of VWF. This causes significantly reduced levels of FVIII in the circulation because of increased clearance of FVIII that is not bound to its carrier protein. It is described as an autosomal recessive form of a mild or moderate form of hemophilia A. FVIII levels may range between 5 and 40 %. Patients with heterozygous 2N VWD do not usually have bleeding symptoms. Bleeding is found in patients that are compound heterozygous of type 1 VWD or, rarely, homozygous for 2N VWD. More than 25 different mutant alleles can cause type 2N disease. The mutations are located in the D/D3 domain of the VWF, which is the region of the protein to which FVIII binds.

Type 3 VWD

Type 3 VWD is an extremely rare disorder with a prevalence of 0.1–5 per million. It is a complete quantitative deficiency with undetectable VWF resulting in a severe bleeding phenotype. Laboratory evaluation reveals undetectable plasma levels of VWF (<2 %) and significant reductions of FVIII in the range of 5–10 %. These patients may develop joint and soft-tissue bleeding, similar to that of patients with hemophilia, in addition to the usual mucocutaneous bleeding symptoms of VWD [14]. Type 3 VWD patients have complete or partial deletion of the VWF gene. More than 100 different mutations have been identified. Those mutations are spread throughout the entire gene.

Diagnosis of VWD

Diagnosis of VWD involves several clinical and laboratory components:

- 1 personal history of mucocutaneous bleeding;
- 2 family history of excessive bleeding; and
- 3 laboratory evaluation that uncovers a quantitative or qualitative defect of VWD.

Bleeding History

Because VWF mediates formation of the platelet plug, VWF results in increased risk for mucocutaneous bleeding symptoms, the clinical characteristic of VWD. The symptoms include nose bleeds (usually occurring 1–5 times/year and lasting >10 min), easy bruising (usually 1–4 per month, atraumatic and >5 cm in diameter), prolonged bleeding from small wounds (>5 min), excessive bleeding after dental procedures (oozing >3 h post extraction), excessive bleeding after surgical intervention requiring surgical re-exploration, and/or genital tract bleeding including menorrhagia or prolonged menstrual bleed.

Children with VWD may experience bruising after immunization or gum bleeding after loss of primary teeth. Musculoskeletal bleeding is rare and most commonly seen in patients with type 3 VWD. Because bruising and nose bleeds may occur in normal individuals, a history of severe recurrent epistaxis or ecchymosis in unusual places or numerous bruises may be more indicative of an underlying bleeding disorder. The presence of iron deficiency in association with blood loss from either recurrent nose bleeds or prolonged menses may be an indicator of an underlying bleeding disorder and this finding should initiate evaluation. Very rarely, VWD may present with petechiae, however this may be related to the use of aspirin or other anti-inflammatory medications by a patient with VWD. Menorrhagia is a frequent presentation in females. VWD has been found in 15–20 % of females with menorrhagia [15]. Routine investigation for VWD in women with isolated menorrhagia is not recommended unless it is associated with additional bleeding symptoms or there is severe menstrual bleeding at menarche [16].

The reporting of bleeding symptoms may be standardized by use of bleeding questionnaires to aid the distinction between normal and abnormal bleeding symptoms [17]. When the ISTH questionnaire is used, a bleeding score of 3 for male patients and 5 for female patients was 98.6 % specific and 69 % sensitive for identification of type 1 VWD [18]. Other quantitative descriptions of bleeding symptoms for patients with type 1 VWD correlated positively with bleeding scores [17–19]. However, for children for whom the hemostatic challenge is minimal, a bleeding

score using adult focused tools is problematic. As a result, the Pediatric Bleeding Questionnaire (PBQ) was introduced in 2009 which included pediatric specific bleeding symptoms, for example umbilical stump bleeding, cephalohematoma, post-circumcision bleeding, post-venipuncture bleeding, and macroscopic hematuria. Use of the PBQ resulted in higher bleeding scores for children with VWD than for normal children [2]. Although bleeding scores are useful tools for quantifying the severity of bleeding symptoms and aiding diagnosis of VWD, they should be used with standardized laboratory evaluation by an expert clinician.

Family History

Because VWD is an inherited disorder most patients will have some evidence of family history consistent with excessive bleeding. For types 1, 2A, 2B, and 2M the disease is a dominant inherited trait. However, because the disease has variable or incomplete penetrance, a convincing family history may be absent. Therefore the lack of a positive family history, particularly for type 1 VWD, does not exclude the possibility of the disease. In addition, types 3 and 2N have a recessive pattern of inheritance and therefore patients may have parents without family history of bleeding and with normal laboratory evaluation.

Laboratory Evaluation

The initial laboratory evaluation of patients with any suspected bleeding disorder should include a complete blood count (CBC), prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, and thrombin time. Laboratory evaluation of VWD requires use of specific assays that reveal the pathophysiology of the disease with either quantitative or qualitative abnormality of the VWF protein. Traditional screening assays are commonly used for initial evaluation of patients with suspected VWD; however several caveats must be taken into account. A CBC may be normal for most of the patients, but may also reveal the presence of iron deficiency anemia resulting in chronic blood loss. Platelet count is mildly reduced in type 2B VWD. aPTT and bleeding time lack sensitivity and specificity and are normal in almost 50 % of patients with mild type 1 VWD. An abnormal aPTT is seen if there is a significant reduction of FVIII or if a second coagulation factor deficiency is also present. A prolonged aPTT may be seen in patients with types 2N or 3 VWD in which FVIII levels may be ≤ 0.10 IU/mL. A normal PTT does not preclude the possibility of VWD. Bleeding time should no longer be used as part of evaluation of suspected VWD. The PFA-100 has been used as a replacement for bleeding

time because of its reproducibility. It is reported to be more sensitive, but less specific, and therefore its use in the diagnosis of VWD remains controversial.

Definitive Assays

Any patient with a history of bleeding and a positive family history should undergo specific testing that measures the amount, structure and function of VWF to achieve a definitive diagnosis. Laboratory tests specific for VWD include:

- 1 measurement of the amount of circulating plasma VWF antigen; and
- 2 a functional test reflecting its ability to aggregate normal platelets in the presence of ristocetin, its ability to bind to collagen, and its ability to bind FVIII.

Von Willebrand Antigen or VWF:Ag

Normal plasma levels of VWF are approximately 1 U/mL or 10 µg/mL (100 %), but a wide range of 0.5–2 U/mL (50–200 %) has been found. Type 1 VWF has a level between 15 and 50 % associated with normal multimer structure. VWF levels in the range 5–20 % tend to be associated with bleeding and are frequently caused by mutation of the VWF gene, and therefore classification of these as type 1 VWD seems appropriate to facilitate their clinical management. In contrast, patients with borderline low levels in the range 35–50 % may have no bleeding symptoms, and rarely have genetic mutations [20]. Diagnosis of type 1 VWD is not useful for such patients, and an assignment of “risk of mild bleeding” may be more appropriate [21, 22]. The exact level of reduction below which a diagnosis of type 1 VWD can be made has been the subject of much debate and continues to be a problem [22, 23]. Over-diagnosis of VWD may have serious implications for individual health insurance and social stigma and this should be considered when making definitive diagnosis. On the other hand, the potential of under-diagnosing VWD for a child for whom the hemostatic system has not yet been challenged sufficiently to manifest bleeding symptoms is also problematic. Because of these challenges and implications, some authors have suggested that the definition of type 1 VWD in children could include *definite* for children with a history of frequent bleeding and low VWF levels, and *possible* for patients with low VWF levels but with no history of frequent bleeding [24]. Also, the suggestion has been made that diagnosis of type 1 VWD be reserved for patients with reduction of VWF levels to <0.15 IU/mL (15 %) but this has not become widely accepted [22].

Ristocetin Cofactor Assay or VWF:RCO

The most common assay used to evaluate VWF function uses the antibiotic ristocetin to induce binding of VWF to platelets and for that reason it is called ristocetin cofactor assay or VWF:RCO. In this assay ristocetin is added to a mixture of patient plasma and formalin-fixed normal platelets. Reduced VWF:RCO in the presence of normal VWF:Ag is indicative of dysfunctional VWF, as is the case in any of the type 2 VWD. A proportional decrease in both the antigen and the function of VWF is indicative of type 1 VWD. The ratio of function to antigen (VWF:RCO-to-VWF:Ag ratio) may be used to differentiate between the type 2 variants.

VWF Binding to Collagen Assay or VWF:CB

Another functional assay is collagen binding of plasma to VWF. VWF binds to three types of collagen, types I, III, and VI. When VWF:CBA is evaluated, the collagen substrates used are type I and III collagen or a mixture of the two (the test will not detect 2M with abnormal binding to type IV collagen). Therefore, VWF:CB results should be interpreted carefully. Most patients with type 2M VWD will have normal collagen binding, because most 2M types are secondary to a mutant VWF that does not bind normally to GP1b, leading to a defective VWF–platelet interactions. Evaluation of VWF:CB should be included in diagnostic investigation of patients with mild bleeding phenotypes suggestive of type 2M [25].

The Ristocetin-Induced Platelet Agglutination (RIPA) Assay

In the RIPA assay a variety of concentrations of ristocetin are added to the patient’s own platelets and plasma. VWF with a “gain of function”, as is observed for type 2B VWD, binds to GP1b on the platelets at very low doses of ristocetin (<0.6 mg/mL), resulting in platelet agglutination. The same is true for patients with platelet-type VWD (a disorder of hyperresponsive platelet because of defects in the platelet GP1b gene), however the distinction between the two is clarified by use of the VWF:RCO with different concentrations of ristocetin, because this test uses formalin-fixed normal platelets. If there is increased activity in response to low doses of ristocetin the defect is in the patient’s VWF and not in the patient’s platelets.

Factor VIII activity or FVIII:C

FVIII coagulant activity should also be evaluated, particularly for patients for whom FVIII levels are low.

If FVIII:C levels are lower than VWF:Ag, strong consideration should be given to 2N VWD, because for these patients the dysfunctional VWF does not stabilize or protect FVIII from cleavage. The level of FVIII may be in the range 0.10–0.4 U/mL.

Multimeric Structure

If an abnormal VWF ratio of function (either VWF:RC₀ or VWF:CB) to antigen (VWF:Ag) is found, the VWF multimeric structure of VWF should be evaluated. Multimeric analysis will reveal the range of VWF multimers in plasma. High-molecular-weight multimers are the most hemostatically active, because they contain the most active binding sites for platelets. Patients with type 2A VWD lack high and intermediate-molecular-weight multimers, whereas type 2B VWD patients lack only intermediate-molecular-weight multimers. As expected, VWF multimers are undetectable for type 3 VWD.

Genetic Testing

Genetic testing does not provide extra useful information in addition to that obtained from good standardized hemostatic testing for VWD. In most cases it does not predict the pathogenic mechanism responsible for the clinical phenotype. Only in special circumstances may use of genetic testing to determine the specific mutation be of clinical relevance, for example:

- (1) In type 1C VWD (Vicenza type) it is useful, because the R1205H variant results in accelerated clearance of VWF. Subjects with this mutation have an exaggerated but short-lived response to desmopressin. Recognition of this variant is important clinically because the short-lived desmopressin response may render this therapy inadequate and require the use of VWF concentrates.
- (2) In type 2A VWD it is useful to determine the existence of the R1597 W variant, which has been associated with increased ADAMTS13-mediated proteolysis.
- (3) It can help discriminate type 2N VWD from hemophilia A carrier status.
- (4) It can help discriminate type 2B VWD from platelet-type VWD.
- (5) It can help distinguish type 2A from type 2M VWD.
- (6) In type 3VWD there are two situations in which molecular genetics provide additional clinical benefit: (a) prenatal testing where a definitive diagnosis may be established from chorionic villus or amniocyte analysis; and (b) to facilitate identification of patients with an increased propensity for anti-VWF antibodies. Patients with partial or total VWF deletions may

be at greater risk of developing antibodies that could inhibit the activity of the replaced VWF. This use must be substantiated with prospective studies [9•].

Genetic testing should not be broadly applied to the diagnosis of VWD, as it is not cost-effective and only provides limited information towards the understanding of the bleeding phenotype of the patient with VWD.

Challenges in the Diagnosis of VWD in children

Because hemostatic testing is often technically difficult, a high-quality and experienced coagulation laboratory and an expert clinician are recommended to avoid misdiagnosis.

Mucocutaneous bleeding symptoms also occur in normal patients and they may be potentially overlooked by patients and physicians. In addition, young children may not have been subjected to a sufficient hemostatic challenge (trauma, surgery, or menarche) to manifest bleeding that would lead to suspicion of the diagnosis. In many instances the family history may not be convincing for a family bleeding tendency. With regard to laboratory evaluation, diagnosis of type 1 VWD remains difficult because VWF levels are affected by external or environmental factors, for example blood type, age, inflammation, stress, pregnancy, hormonal cycles, and a variety of medications.

- (1) Delays in processing the sample and exposure to extreme temperatures can lead to artificially low VWF levels. This is of great concern for centers that do not process the sample immediately but send it to other laboratories [26].
- (2) Levels of VWF increase 1–2 % with each year of age. Thus, for many patients with initial low levels of VWF of approximately 30 % a progressive increase in levels, perhaps to normal levels, will occur by middle age [27].
- (3) Diagnosis for neonates is challenging because the stress of vaginal delivery increases VWF levels [28, 29].
- (4) Plasma levels of FVIII and VWF increase twofold to fivefold during physiologic stress, for example fainting and exercise [30]. For children with mild disease the stress of anticipation of phlebotomy may be sufficient to increase VWF levels to normal.
- (5) Increased level of thyroid hormones is associated with increased VWF levels whereas the hypothyroid state results in low levels [31–33].
- (6) VWF levels may be lowest during menses and therefore the timing of the testing during the menstrual cycle is of relevance when interpreting results. Testing during the first three days of the menstrual cycle is recommended [34].

- (7) 15 % of patients with blood group O have VWF levels <50 %. VWF:Ag and FVIII levels are approximately 25 % lower in patients with blood group O than in patients with group A, B, or AB [35].

All of these factors must be taken into consideration when evaluating a patient with suspected VWD, particularly type 1. It is important to consider personal and family history of bleeding and laboratory evaluation in diagnosis of VWD and to repeat the laboratory evaluation at least twice to confirm or exclude diagnosis of VWD [24]. Primary care practitioners should refer patients suspected of VWD to specialized centers that have medical expertise and a hemostasis laboratory on-site.

Management

The treatment options for patients with VWD depend on accurate diagnosis of the disorder. Treatment options and prophylaxis vary depending on subtype (Table 2). In general, localized measures to control bleeding are recommended. These measures include direct pressure to the site of bleeding or injury, biting down on a piece of gauze may halt bleeding from a tooth socket, application of a compression bandage and cold pack to an injured limb may reduce hematoma formation. For prolonged nose bleeds, a stepwise approach that starts with indirect pressure and then escalates to packing and subsequently to nasal cautery may be required. Application of fibrin glue to the sites of bleeding may be helpful.

Other adjunctive therapy is useful for injuries that involve the oral and nasal mucosa. Antifibrinolytic therapy with epsilon aminocaproic acid (EACA) or tranexamic acid is generally used. Antifibrinolytic therapy is used alone or as adjuvant to replacement therapy. EACA is available in oral and intravenous formulations. The oral formulation is a 500-mg capsule or a liquid with a concentration of 1.5 g/5 mL. The oral dose is 100 mg/kg (maximum of 6 g) as loading dose followed by 50 mg/kg (maximum of 3 g). It can also be effectively used topically as a mouthwash at a dose of 10 mL (1.5 g/5 mL) four times per day. For patients with menorrhagia, administration of estrogen could elevate plasma levels VWF and FVIII.

Desmopressin

Desmopressin (1-diamino-8-D-arginine Vasopressin or DDAVP), is a vasopressin analog that promotes release of VWF and FVIII from storage sites into the circulation. Its administration increases plasma VWF and FVIII levels by approximately twofold to eightfold within 1–2 h of administration. Because individual response to desmopressin is

variable, a trial with laboratory measurement of VWF and FVIII at baseline and 1, 2, and 4 hours after administration is advised [36]. An individual usually responds similarly to repeated doses, thus the plasma level of VWF achieved in the trial predicts response to future therapy. For instance, a patient with VWD who achieves 40 % VWF activity would be safe taking desmopressin for tooth extraction, menorrhagia, or a minor surgical procedure but not for treatment of life-threatening hemorrhages or major surgery, which requires a level of at least 80 %. For this reason, documentation of an adequate response to desmopressin is of critical importance.

Highly concentrated intranasal desmopressin preparation (Stimate) is most commonly used. Stimate delivers 150 µg/spray (a much higher concentration than that found in the nasal spray to treat enuresis). The dose for children weighing less than 50 kg is 150 µg; it is 300 µg for larger children. Desmopressin can also be administered intravenously [37]. The usual intravenous dose is 0.3 mg/kg (maximum 20 µg) infused in 50 mL normal saline over approximately 30 min. The peak effect is achieved within 30 and 90 min for the intravenous and intranasal routes, respectively. Desmopressin is safe and well tolerated. Common side effects include facial flushing and headaches. Some patients may experience tachycardia and mild reductions in blood pressure with IV infusions and, therefore, it is best to administer with patients lying down. The most serious side effect is severe hyponatremia and seizures related to the antidiuretic effect of the drug [38]. Reduction of fluid intake for 24 h is an important precaution to prevent water intoxication, particularly for children younger than 3 years of age. Because of the unreliability of control of fluid intake for young children most practitioners do not use desmopressin for children less than 2 years of age.

The main limitation of the use of desmopressin is the development of tachyphylaxis with repeat administration. VWF and FVIII levels often fall to approximately 70 % of that documented with the initial dose when given at repeated intervals of less than 24 h. However, if two days have elapsed between doses, a response similar to baseline can be expected.

Most patients with type 1 VWD respond to desmopressin, but some with severe type 1 VWD may not respond. For patients with type 2 VWD, desmopressin may help mild bleeding, but severe bleeding requires replacement therapy. Its use to treat type 2B VWD is regarded as contraindicated, because of the transient decrease in platelet count that follows release of the mutant VWF, however, some patients may have adequate hemostasis and, therefore, it can be used in individual cases [39]. For types 2M and 3 there is, typically, an inadequate response to desmopressin because of the respective loss-of-function

Table 2 Laboratory evaluation and treatment options

VWD type	Laboratory evaluation	Treatment options		
		Desmopressin	VWF/FVIII concentrate	Platelet
Quantitative Deficiency				
1	VWF:Ag ↓ VWF:RCo ↓ RCo:Ag >60 % FVIII nl or ↓ Multimer normal	Treatment of choice	If desmopressin is not effective	Not indicated
3	VWF:Ag ↓↓↓ VWF:RCo ↓↓↓ RCo: Ag – FVIII <10 % Multimer –	Not indicated	Required for major bleeding and surgery	Indicated for persistent bleeding
Quantitative deficiency				
2A	VWF:Ag ↓ VWF:RCo ↓↓ RCo:Ag < 60 % Multimers: Low HMW and intermediate RIPA: ↓	Transient response	Required for major bleeding and surgery	Not indicated
2B	VWF:Ag ↓ VWF:RCo ↓↓ RCo:Ag <60 % Multimers abnormal RIPA: ↑ Thrombocytopenia	May cause drop in platelet count	Required for major bleeding and surgery	Indicated if thrombocytopenia remains severe after VWF replacement
2M	VWF:Ag ↓ VWF:RCo ↓↓ RCo:Ag < 60 % FVIII 10–40 % Multimer normal	Partial response for minor bleeding	Required for major bleeding and surgery	Not indicated
2N	VWF:Ag ↓ VWF:RCo ↓ RCo:Ag >60 % Multimer normal FVIII < 10 %	May result in partial but transient response FVIII increase for ~3 h	Required for major bleeding and surgery	Not indicated

VWF:Ag von Willebrand antigen, VWF:RCo ristocetin cofactor assay, RCo:Ag VWF:RCo/VWF:Ag ratio, RIPA ristocetin-induced platelet agglutination assay, FVIII factor VIII

and complete absence of VWF protein. For type 2N, desmopressin increases VWF and FVIII levels by two to ninefold; however FVIII increases for an average of 3 h. Thus for patients with type 2N, desmopressin should be used when a very transient rise in FVIII is required [40]. Because of the variability of response to DDAVP, response data from a DDAVP challenge test could be used to overcome phenotypic assay limitations. For instance, for type 1 VWD, VWF and FVIII rise after DDAVP, and the

RCo/Ag ratio remains >0.7, but for type 2A the RCo/Ag ratio remains <0.7 [41•].

Replacement Therapy

Replacement therapy is used to treat major surgery, trauma, or life-threatening bleeding, and is also considered for treatment of minor bleeding, for example epistaxis, menorrhagia, lacerations, tooth extractions, and minor surgery

for selected patients for whom repeated doses of desmopressin do not achieve adequate hemostasis.

Intravenous treatment with plasma-derived concentrates of normal VWF and FVIII, for example cryoprecipitates, was often used 30–40 years ago, but it is not viral attenuated and therefore, not indicated for use to treat VWD unless other products are unavailable. Today, several commercial plasma-derived VWF/FVIII concentrates are available, but only two, Humate-P and Alphanate, are currently FDA-approved for treatment of patients with VWD disease [42]. Dosing recommendations currently are made in VWF:RCO units/body weight [43]. A dose of 1 to 1.5 VWF:RCO units/kg will increase the plasma VWF level by 2 % and 1 FVIII unit/kg will increase the plasma FVIII level by 2 % [44, 45]. Repeat infusion can be given every 12–24 h depending on the clinical situation. Measurement of VWF:RCO and FVIII levels in patients receiving repeat infusions is recommended, not only to ensure adequate hemostasis, but also to monitor for supra physiologic levels of FVIII than can increase the potential risk of thrombosis.

It is important to remember the hemostatic level that must be achieved and the half-life of a transfused protein to successfully treat or prevent bleeding. Based on treatment practices of hematologists in the United States, maintaining VWF and FVIII activity of 40–50 % for 1–3 days is sufficient for minor bleeding. However, for major surgery or life-threatening bleeding, an initial level of 80–100 % is desired followed by 50 % for at least 3 days, but FVIII should be maintained above 50 % for at least 5–7 days and above 30 % for an additional 5–7 days to prevent late bleeding [46].

Finally, in the rare event that infusion of an intermediate purity concentrate is ineffective at stopping bleeding, transfusion of a platelet concentrate is beneficial, presumably because it facilitates delivery of a small amount of VWF (contained in normal platelets) to the site of vascular injury [47, 48].

Recombinant VWF

A highly pure recombinant human VWF (rVWF) has been developed in a genetically engineered Chinese hamster ovary cell line that co-expresses VWF and FVIII genes. The highly pure (>99 % purity) rVWF product has a homogeneous and intact VWF multimer distribution, because it is not exposed during manufacture to the VWF protease ADAMTS13. A recent phase 1, multicenter, randomized clinical trial by Mannucci and associates investigated the safety and pharmacokinetics (PK) of rVWF combined at a fixed ratio with recombinant factor VIII for subjects with type 3 or severe type 1 von VWD. The trial proved that rVWF was well tolerated and that the PK of

rVWF:RCO, VWF:Ag, and VWF:CB were similar to those of the control plasma-derived VWF and FVIII. The observed enhanced stability of endogenous FVIII further supported the concept of use of rVWF alone for treatment of VWD to maintain sufficient VWF and FVIII activity for an extended period of time. This property of rVWF is potentially useful for use in clinical situations, for example the post-operative period and continuous prophylaxis, however, more studies in larger clinical trials are necessary to validate this initial finding [49].

Gene Therapy

The development of gene-delivery approaches has led to the possibility of cure for VWD. Gene therapy can potentially provide constant, sustained VWF synthesis for patients with VWD, obviating the need for repeated injection and the risk of viral infections associated with plasma-derived VWF. Ex-vivo gene therapy involves isolation of cells from the patient followed by expansion and genetic modification in culture with vectors expressing VWF and subsequent re-administration of the engineered cells to the patient. In-vivo gene delivery involves administration of a gene-transfer vector encoding VWF directly to the patient, which leads to in-situ genetic modification of the target cells. Gene therapy for VWD requires use of a gene-delivery system that is efficient, safe, non-immunogenic, and enables long-term gene expression. Several important lessons have been learned from ongoing VWD gene therapy research, including limitations and advantages of particular viral (retroviral, adenoviral, and adeno-associated viral) and non-viral vectors, and determination of the “target cells” (endothelial cells, megakaryocytes, and/or hepatocytes); these have enabled refinement of gene therapy techniques. Although there has been much progress in VWD gene-therapy approaches, they are still in their early stages. Their efficacy must be demonstrated in large animal models before they move to clinical trials with VWD patients, and the results must compare favorably with existing protein-replacement therapy [50].

Conclusions

Von Willebrand is the most common inherited bleeding disorder. It is caused by genetic mutation in the VWF gene. It is classified into three primary subtypes. Type 1 includes partial quantitative deficiency, type 2 (A, B, M, and N) includes qualitative defects, and type 3 includes virtually complete deficiency of VWF. Diagnosis of VWD remains a challenge, particularly among the pediatric population,

among whom a hemostatic challenge may not have occurred. Interpretation of the symptomatology and the results from laboratory evaluation requires an experienced clinician to accurately diagnose VWD and provide adequate treatment.

Disclosure Karen S. Fernández and Pedro A. de Alarcón declare that they have no conflict of interest.

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