Low von Willebrand factor: sometimes a risk factor and sometimes a disease

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A sufficiently low level of von Willebrand factor (VWF) predisposes to bleeding that can be quite serious, and low VWF is a diagnostic feature of von Willebrand disease (VWD) type 1, which is characterized by partial quantitative deficiency of VWF. Recent groundbreaking studies of patients with VWD type 1 have delineated several pathophysiologic mechanisms that determine the plasma concentration of VWF, but the relationship between VWF level and the likelihood of bleeding remains less well understood. In part, this problem reflects the broad range of VWF levels in the population, so that the distinction between "normal" and "low" is arbitrary. The risk of bleeding certainly increases as the VWF level decreases, but the relationship is not very strong until the VWF level is very low. Furthermore, mild bleeding symptoms are common in apparently healthy populations and have many causes other than defects in VWF, which can make it impossible to attribute bleeding to any single factor, such as low VWF. These difficulties might be resolved by an epidemiologic approach to VWF and other risk factors for bleeding, analogous to how physicians manage multiple risk factors for cardiovascular disease or venous thromboembolism.

Determinants of Plasma VWF Concentration, Structure, and Function

The biosynthesis and catabolism of von Willebrand factor (VWF) provide a framework for understanding how VWF mutations affect plasma VWF levels. VWF is made in endothelial cells and megakaryocytes, starting with a 350 kDa VWF precursor that consists of several structural motifs arranged in the order D1-D2-D′-D3-A1-A2-A3-D4-B1-B2-B3-C1-C2-CK. This proVWF species dimerizes "tail-to-tail" in the endoplasmic reticulum through disulfide bonds between the C-terminal CK domains. ProVWF dimers are transported to the Golgi apparatus, where the propeptide (D1D2) is cleaved and additional disulfide bonds form "head-to-head" between D3 domains to produce gigantic linear multimers that are packaged as tubular arrays into secretory vesicles. In endothelial cells, VWF multimers are packaged into rod-shaped vesicles called Weibel-Palade bodies, and almost all the protein content of Weibel-Palade bodies consists of VWF. In platelets, VWF is stored in α-granules with other hemostatic proteins. Platelet VWF comprises approximately 15% of circulating VWF.[1]

After secretion into the blood, VWF is subject to proteolysis and clearance. The size of newly secreted VWF multimers has not been determined, but the largest circulating multimers may have a mass of at least 20 million Da and contain at least 80 VWF subunits. The metalloprotease ADAMTS13 cleaves these VWF multimers when they experience enough tensile force to unfold the VWF A2 domain and expose the Tyr1605-Met1606 bond to the enzyme. Cleavage occurs under conditions of high fluid shear stress in the microcirculation or pathologically at sites of arterial stenosis, and cleavage is facilitated when VWF multimers bind to cell surfaces or interact with platelets.[2,3] As a consequence of this proteolytic remodeling, circulating VWF multimers are smaller than those secreted initially by endothelial cells or platelets.

VWF multimers appear to be cleared from the circulation mainly by macrophages in the liver and spleen in a process that is independent of multimer size.[4,5] The rate of VWF clearance depends on blood type-specific glycosylation patterns. For example, VWF is one of very few plasma proteins that bear ABO blood group antigens attached to N-linked oligosaccharides. Blood type O is associated with VWF levels approximately 25% lower than the population average, and the mean half-life of VWF is approximately 10 hours for type O subjects, substantially shorter than the half life of 25.5 hours for other ABO blood types.[6] VWF level is also approximately 20% higher in persons homozygous for the *Secretor* (*Se*) allele, suggesting that *Secretor* oligosaccharides on VWF influence its clearance.[7] The

mechanism of oligosaccharide-dependent VWF clearance is unknown.

The plasma concentration of VWF is not controlled very tightly, and the range of values observed across the population is very broad. The population mean VWF level is 100 IU/dL and 95% of values lie between approximately 50 and 200 IU/dL (**Figure 1**).[1]

Pathogenesis of VWD Type 1

As might be expected from the complexity of VWF biosynthesis and catabolism, several mechanisms have been identified that reduce the plasma concentration of VWF while preserving approximately normal VWF function and multimer structure, which are characteristic features of von Willebrand disease (VWD) type 1. A few patients are heterozygous for deletion, frameshift, nonsense, or other VWF null mutations that reduce the synthesis of VWF.[8] However, VWD type 1 is usually caused by single amino acid substitutions that appear to have a dominant negative effect on VWF levels. As noted in a recent review,[9] 75% of the approximately 144 VWD type 1 mutations listed in the ISTH VWD database (www.vwf.group.shef.ac.uk) are missense mutations; the remainder are nonsense, splice site, frameshift or deletion mutations expected to cause a null allele. The reciprocal pattern is observed for VWD type 3: of 107 reported mutations, 80% are expected to cause null alleles.

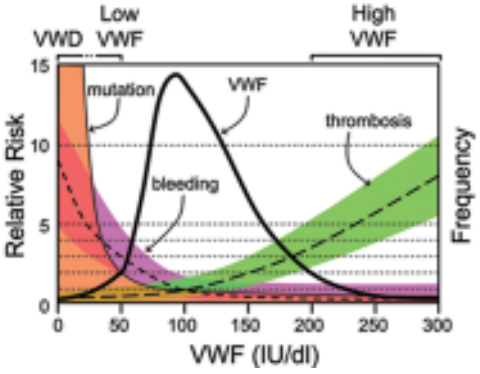

Figure 1. Model for the relationship of von Willebrand factor (VWF) level to risk of bleeding, thrombosis, and *VWF* mutations. The thick solid line indicates the frequency distribution of VWF levels (IU/dL) for the population, and 95% of values lie between 50 IU/dL and 200 IU/dL. Also shown are estimates of the relative risk of bleeding (short-dashed line, magenta shading), thrombosis (long-dashed line, green shading), and mutation within the *VWF* gene (thin solid line, orange shading) as a function of VWF level; the relative risk is defined as 1.0 at the population mean VWF level of 100 IU/dL. As indicated at the top, VWF levels < 20 IU/dL are generally consistent with a diagnosis of VWD type 1, low VWF levels (30 to 50 IU/dL) confer a modest risk of bleeding, and high VWF levels (> 200 IU/dL) confer a modest risk of thrombosis.

The preponderance of missense mutations in VWD type 1 is one of the most interesting and surprising results of three major studies from the European Union,[10-14] Canada[15,16] and the United Kingdom.[17,18] Some of these missense mutations have been shown to impair the intracellular transport of VWF subunits, and others lead to rapid clearance of VWF from the circulation.[14,19] Patients with such mutations usually have plasma VWF levels less than 20 IU/dL, significant bleeding symptoms, and a strongly positive family history of bleeding.[11,12,15,16]

VWD Type 1 or Low VWF

Patients with VWF levels less than 20 IU/dL almost always have a dominant-negative *VWF* mutation (**Figure 1**) and significant bleeding symptoms. The predictable clinical phenotype and high heritability justify a diagnosis of VWD type 1. More commonly, patients have modestly low VWF levels (eg, 30 to 50 IU/dL) that pose significant problems for diagnosis and treatment because such VWF levels are common, bleeding symptoms are common, and a modestly low VWF level is only a modest risk factor for bleeding.

Low VWF Levels Are Common

A VWF:Ag or VWF:RCo level below 50 IU/dL has often been used as a criterion for diagnosing VWD type 1, but this practice places 2.5% of the population at risk for a diagnosis of VWD type 1 compared to the estimated prevalence of approximately 0.002% to 0.01% for all symptomatic VWD seen in specialized treatment centers.[1] The difference between these values might conceivably be explained by underdiagnosis of medically significant VWD. However, the size of the discrepancy—two to three orders of magnitude—suggests instead that a low VWF level is insufficient to identify patients who may bleed significantly, much less to diagnose VWD.

In addition, the heritability of VWF levels across the population distribution is low. Only 25% to 32% of the variance in VWF levels appears to be heritable in family studies.[20,21] The ABO blood group locus is the only modifier of VWF levels unlinked to the VWF gene that is readily identified in population studies, and it accounts for approximately 20% to 30% of the total heritable variance.[22] Therefore, most of the variation in VWF level is not heritable, and known genes explain little of the variation that is heritable.

The *VWF* gene has surprisingly little influence on VWF levels in the general population. A study of sibling pairs found that the VWF locus could account for 20% of the variation in VWF level,[23] but another genome-wide linkage study did not observe this relationship.[22] However, among patients diagnosed with VWD type 1, the likelihood of

linkage to the *VWF* gene varies inversely with VWF level. If the index case has VWF:Ag less than 20 IU/dL, affected pedigrees almost always show cosegregation of a low VWF level and a *VWF* gene mutation (**Figure 1**).[11,12,15,16] Persons with less striking VWF deficiency exhibit less predictability, approaching the behavior of the healthy population. In the Canadian study of VWD type 1, a *VWF* mutation was found in just 49% of index cases with VWF:Ag between 30 and 50 IU/dL.[16] In the European study, linkage to the *VWF* locus was demonstrated in only 51% of similar cases and decreased further when subjects with abnormal multimer patterns were excluded.[11] Thus, even for subjects selected from hemostasis clinic populations, a modestly low VWF level may not be attributable to a *VWF* gene defect.

Bleeding Symptoms Are Common

Surveys have repeatedly shown that healthy persons often report mild bleeding including epistaxis (in 5% to 23%), gum bleeding (in 7% to 47%), easy bruising (in 12% to 50%), bleeding from minor cuts or abrasions (in 0.2% to 33%), bleeding after tooth extraction (in 5% to 42%), excessive postoperative bleeding (in 1.4% to 28%), postpartum bleeding (in 6% to 23%), and menorrhagia (in 23% to 68%).[1] Responding to a standardized bleeding questionnaire, 23% of healthy controls reported at least one such bleeding symptom, compared with more than 88% of patients with a diagnosis of VWD type 1,[10,24] but none of these symptoms is specific to VWD.

The European study of VWD type 1 also employed a standardized questionnaire and bleeding score to evaluate the severity of bleeding symptoms. The average bleeding score was significantly different for subjects with VWD type 1 index cases and unaffected family members, and there was an inverse relationship between the bleeding score and VWF level.[10,24] However, all groups displayed a broad overlapping range of bleeding scores and, surprisingly, the bleeding score of the index case did not predict the bleeding score for affected family members.[10,11] In fact, the bleeding score was not clearly linked to the *VWF* gene.[11] On the other hand, for the prediction of postoperative bleeding, a high bleeding score was superior to a low VWF or factor VIII level.[10]

Many Diagnoses of VWD Type 1 Are False Positives

The European VWD type 1 study suggests that past bleeding is a better guide to future bleeding than is laboratory testing for VWF. However, this study population was selected for a strong family history of bleeding, and the dissociation of bleeding and low VWF might be explained if factors unrelated to low VWF were important in the pathogenesis of bleeding, as proposed by the authors.[10] Variable expressivity of VWD type 1 might explain the occurrence of low VWF without bleeding, but this explanation cannot account for the occurrence in the same pedigree of persons who bleed despite having normal VWF levels. Instead, the inheritance pattern suggests that bleeding and low VWF are independent. As a corollary, if family members can bleed with normal VWF levels, then the index case also may bleed independent of their low VWF level, perhaps because of a distinct inherited disorder, or perhaps coincidentally because bleeding symptoms are common.

The likelihood that bleeding and low VWF associate by coincidence has been estimated for healthy populations.[25] Because personal bleeding symptoms are common, a family history of bleeding often occurs by chance. Considering average families of four persons, a 25% probability of having any bleeding symptom[24] implies that 14% of all family members will have both a personal and family history of bleeding. The 2.5% prevalence of low VWF (by definition) then implies that 0.4% of the population will have bleeding, low VWF, and a family history of bleeding—common criteria for diagnosing VWD type 1—solely by chance. This estimated 0.4% prevalence of potentially false-positive VWD type 1 is similar to the prevalence of 0.8% for VWD type 1 upon applying similar criteria to 1218 schoolchildren in Italy, or 1.3% for 600 schoolchildren in the United States, or 0.6% for 832 patients screened preoperatively. This background of coincidence may account for the consistent findings from the Canadian,[16] European[10,11] and UK[18] studies of VWD type 1, that when the VWF level is modestly decreased (eg, 30 to 50 IU/dL), bleeding symptoms and VWF level are often inherited independently,[10,11,16] and neither symptoms nor VWF level exhibit consistent linkage to the *VWF* locus.[11,16,18]

Moderately Low VWF Is a Modest Risk Factor for Bleeding and Protects Against Thrombosis

If moderately low VWF levels conferred a significant risk of medically important bleeding then measuring plasma VWF might be clinically useful, whether or not low levels were heritable or caused by *VWF* mutations. However, the available data suggest that moderately low VWF carries a small risk of bleeding. For example, bleeding histories and VWF levels were obtained from published reports for 191 obligate heterozygous relatives of patients with VWD type 3. The geometric mean VWF level was 47 IU/dL, with a range (±2 SD) of 15 to 140 IU/dL. The VWF level was less than 50 IU/dL in 117 patients and 31 of them (26%) had mild bleeding symptoms including bruising, epistaxis, menorrhagia, and bleeding after tooth extraction; VWF was more than 50 IU/dL in 74 patients and 10 (14%) had bleeding symptoms including one instance of postoperative bleeding. Therefore the relative risk of any bleeding, given

a low VWF level, was approximately 1.9 ($P = 0.046$, Fisher's exact test). There was a trend for increased bleeding at the lowest VWF levels. Among 31 persons with VWF less than 30 IU/dL, 12 (39%) had symptoms.[25]

Two studies of healthy teenagers also indicate that modestly low VWF levels increase the risk of mild bleeding. A case-control study in Iceland recorded bleeding symptoms for 63 (7.8%) of 809 teenagers (15 to 16 years old). Low values of VWF:Ag were found in 10 (20.4%) of 49 subjects with bleeding symptoms, compared to 10 (6%) of 166 controls without bleeding symptoms (odds ratio [OR] 4.0, 95% confidence interval [CI] 1.6-10.4). Abnormal platelet aggregation studies were found in another 12.8% of teenagers with bleeding, compared to 4.4% of controls. Therefore, marginally low VWF levels or mild platelet function defects were found in roughly one third of teenagers with bleeding symptoms.[26] In a similar study of girls (mean age 18 years old) from Malmö, Sweden, VWF levels in the bottom 2.5 percentile were found in 17 (9.7%) of 176 subjects with bleeding symptoms, and in 1 (1.4%) of 70 subjects without bleeding symptoms (OR 7.4, 95% CI 0.96-56). None of these girls met criteria for a diagnosis of VWD because their VWF levels were only slightly decreased, the bleeding was too mild, or a family history of bleeding was absent.[27]

As an alternative to population screening, the relationship between VWF and bleeding can be assessed in patients with specific symptoms. For example, menorrhagia is the most common symptom attributed to VWD, and three studies have investigated the prevalence of VWD among women seen in gynecology clinics with menorrhagia. Two studies used an alkali hematin assay to measure menstrual blood loss, and one used a pictorial chart score. All three studies defined VWD simply by a low VWF level at least 2 SD below the mean. Among 218 women with objectively documented menorrhagia, 19 (8.7%) had low VWF. In these studies low VWF occurs by definition in 2.5% of the population, so the relative risk of menorrhagia for women with low VWF would be approximately 3.9,[25] although the true magnitude of the risk is uncertain because of potential biases in patient selection and assumptions made for this analysis.

An increased risk of bleeding is not limited to subjects with VWF levels more than 2 SD below the mean. One of the most interesting findings of the European VWD type 1 study was that the severity of bleeding varied inversely with VWF across the entire population distribution of VWF levels. An inverse correlation was found between all quartiles of the bleeding score and levels of VWF:RCo, VWF:Ag, and factor VIII, with mean interquartile differences of 14, 13, and 11 IU/dL, respectively.[10]

The inverse relationship between VWF and bleeding appears to be counterbalanced by a direct relationship between VWF and thrombosis, mediated partly through associated changes in factor VIII levels. For example, VWF level correlates with the risk of venous thromboembolism,[28,29] thromboembolic stroke, and acute myocardial infarction.[30-33] In each case the OR is approximately 2 to 5 for comparison of the highest and lowest quartiles of VWF level, which is similar to the OR for bleeding with low VWF. These common thrombotic events associated with high VWF are more likely to cause death acutely than is bleeding associated with modestly low VWF. In addition, a recent case-control study found that VWF level predicted long-term mortality after ischemic stroke: for the highest and lowest quartiles of VWF, 5-year survival was 25% and 70%, respectively.[34] Unfortunately we cannot easily adjust our VWF levels at present, but if we could then women might select a high VWF level to protect against menorrhagia, with an option to pick a low VWF level after menopause to protect against cardiovascular and thromboembolic events.

Managing VWD Type 1 and Low VWF

A diagnosis of VWD type 1 is useful for patients with very low VWF levels (< 20 IU/dL), dominant negative VWF mutations and significant bleeding. These characteristics usually are transmitted together in the family,[11,12,15,16] and labeling this constellation of findings as a disease makes sense for the physician and for the patient. Bleeding in the skin, nasal mucosa, reproductive tract and other sites can be attributed confidently to the very low VWF level and treated accordingly.

As the VWF level increases toward the normal range, however, a modest risk of bleeding decreases continuously, and no bright line separates binary categories of "diseased" and "healthy" persons. In addition, if persons with VWF levels above 30 IU/dL have bleeding symptoms, their symptoms and low VWF level usually do not cosegregate in their families, and *VWF* gene mutations occur in a minority of them.[11,16,21] Given the lack of heritability, diagnosing VWD type 1—an inherited disorder—is usually unhelpful. Nevertheless, low VWF near the bottom end of the population distribution is a risk factor for mild bleeding,[10,25-27] and it can be managed as such. Based in part on data from the Canadian, European and UK VWD type 1 studies, I can propose some rules of thumb.

The Value of VWF Testing Depends on How Patients Are Ascertained

Finding a modestly low VWF level has almost no utility for an asymptomatic person, whether they are identified by screening of healthy populations, through the investigation

of families with VWD, or discovered incidentally. In the absence of a bleeding history, a modestly low VWF level does not predict significant bleeding in the future.[10,11] Therefore, unless there is a compelling reason, VWF levels should not be checked in asymptomatic persons.

Bleeding Symptoms Are More Important Than VWF Level

On the other hand, testing of patients who seek medical attention because of bleeding will identify the relatively small number with clinically severe VWD type 1, type 2 and type 3, as well as a much larger number with a moderately low VWF level. For example, approximately 13% of women with menorrhagia have VWF levels less than 50 IU/dL, compared with approximately 2.5% of healthy controls.[25] However, family studies show that bleeding and moderately low VWF levels do not cosegregate reliably. In fact, a history of bleeding is a reasonably good predictor of future bleeding, whereas a VWF level of 30 to 50 IU/dL is not.[10] Ascertainment based on bleeding symptoms identifies patients who are likely to bleed in the future, independent of modest decreases in VWF.

Most Bleeding Cannot Be Explained by Laboratory Testing

Primary hemostasis depends on many factors besides VWF that affect how platelets interact with and respond to sites of vascular injury. Defects in any of these factors, known or unknown, may cause bleeding. Given this complexity, we should not be surprised that laboratory testing identifies a plausible hemostatic defect in a minority of patients evaluated for abnormal bleeding that appears to be hereditary. In typical studies, some patients (3% to 19%) have low VWF levels, some (9% to 24%) have poorly defined platelet function defects, and some (4% to 22%) have mild clotting factor deficiencies, but most (47% to 69%) have normal test results.[35] Using available laboratory tests, it appears that we will never know why most patients bleed.

If a Patient with Modestly Low VWF Bleeds Severely, Look for Other Causes

The association of a low VWF level with bleeding does not necessarily reflect causation. By itself, a VWF level of 30 to 50 IU/dL is a weak risk factor for mild bleeding, and the occurrence of severe bleeding should prompt the physician to consider other defects that might cooperate with a low VWF level or independently cause bleeding. For example, a study of VWD type 1 in Spain found that two symptomatic patients had both low VWF and mild factor XI deficiency.[36] Several candidate gene haplotypes were investigated in a subset of patients from the European VWD type 1 study, and the severity of bleeding was linked to specific DNA markers for platelet membrane proteins $\alpha 2\beta 1$, $\alpha IIb\beta 3$, and GPVI.[37] Another patient in the European VWD type 1 study had the VWF mutation L1774S, but the significance of this sequence change is uncertain because the patient had normal values for VWF:Ag (64 IU/dL) and VWF:RCo (97 IU/dL), and a normal VWF multimer pattern. These results do not support a diagnosis of VWD, and further study showed that the patient had a loss-of-function K174E mutation in the platelet $P2Y_{12}$ ADP receptor that may account for bleeding.[38]

An interesting study of 32,463 pregnant women found that 1269 of them (4%) developed non-severe postpartum hemorrhage (treated satisfactorily with prostaglandin infusion), and 317 (1%) of them developed severe postpartum hemorrhage (decrease in hemoglobin ≥ 4 g/dL or transfusion of ≥ 4 units of packed red cells). Based on testing 6 to 9 months after delivery, risk factors for severe postpartum hemorrhage included low (but not deficient) levels of fibrinogen, VWF, factor XI, platelet GPIbα, activation-induced exposure of platelet $\alpha IIb\beta 3$, and several other variables. At least two risk factors were present in 1.6% of controls, 3.5% of non-severe postpartum hemorrhage, and 20.8% of women with severe postpartum hemorrhage. Thus, the interaction of several mild hemostatic risk factors appears to increase the incidence of severe postpartum hemorrhage.[39]

Treat Moderately Low VWF as a Risk Factor, Not as a Disease

Among the US population of about 350 million, 8.75 million (2.5%) will have VWF levels less than 50 IU/dL, but at most only 35,000 (100 per million) have been diagnosed with any type of VWD, of whom perhaps 26,000 would have a diagnosis of VWD type 1 (about 75% of the total).[1] Misdiagnosis of major bleeding is unlikely to explain the difference between 26,000 patients with VWD type 1 and 8.75 million persons with low VWF. Instead, it seems likely that most persons with low VWF never bleed, and that bleeders differ from nonbleeders because they have other risk factors that interact with low VWF to increase the risk of bleeding. This conclusion is consistent with studies, discussed above, showing that hemostatic risk factors in addition to low VWF occur in some patients with significant bleeding.

In this respect, bleeding is analogous to myocardial infarction or venous thromboembolism, which often occur in subjects with several individually weak risk factors that cooperate to cause an event. We routinely use a data-driven risk management strategy for cardiovascular disease and venous thromboembolism, and application of such an approach to bleeding is limited only by the available data on the risks and benefits of treatment. A promising approach has been described using Bayesian analysis of

bleeding symptoms, VWF level, and linkage to the *VWF* gene to predict the likelihood of VWD type 1 among subjects enrolled in the European VWD type 1 study.[13] Extension of this method to patients newly referred for diagnostic evaluation would be extremely helpful to characterize the risks associated with low VWF and the potential benefits of treatment. In particular, the risk of bleeding as a function of VWF level needs better definition, so that we can determine whether a specific patient would benefit most from a risk management approach to their low VWF level, or an explicit diagnosis of VWD type 1.

For now, patients with modestly low VWF and bleeding symptoms who do not meet criteria for a diagnosis of VWD type 1 may be considered to have "low VWF" that slightly increases their risk of mild bleeding, just as we tell other patients that "high blood pressure" may increase their risk of cardiovascular events. For patients with low VWF, empiric therapy with desmopressin (DDAVP) to raise the VWF level can be used to treat bleeding or to prevent bleeding by prophylactic treatment in some circumstances. Burdening them with a diagnosis of VWD type 1 does not seem useful because a minority will have a VWF mutation, low VWF and bleeding are unlikely to cosegregate in their families, and any bleeding may have several causes—known or unknown—besides low VWF.

Disclosures

Conflict-of-interest disclosure: The author is a consultant for Ablynx and Baxter BioSciences and is on a Baxter BioSciences advisory board.
Off-label drug use: None disclosed.

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