VON WILLEBRAND DISEASE

A Discussion for Clinicians

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Introduction

Von Willebrand disease (VWD) is an autosomally-inherited congenital bleeding disorder in which there is a deficiency or dysfunction of von Willebrand factor (VWF). VWF is a multimeric glycoprotein made in endothelial cells and secreted into the plasma, and also made in megakaryocytes and present in platelets. VWF has two major functions, as follows: (1) it attaches to subendothelial collagen and to platelets, resulting in a platelet plug at the site of injury of small blood vessels, and (2) it binds and transports factor VIII (FVIII). The predominant clinical problems are nosebleeds, excessive bleeding from small skin cuts and from lesions in the mucosa or gastrointestinal tract, excessive menstrual bleeding, and excessive bleeding after trauma, surgical operations or childbirth. Some patients with severe deficiencies of VWF also have severe deficiencies of FVIII and may bleed into joints or muscles, like patients with hemophilia A.

Estimates of the prevalence of VWD vary widely, as high as 1 in 100 or 1 in 1000 persons, based on solicited histories and laboratory tests. Another estimate of 1 in 10,000 persons includes only persons actively seeking treatment. The prevalence of severe VWD can be determined fairly accurately; it is highest in Sweden (one case per 200,000 persons) and its neighboring countries, and in countries where consanguineous marriages are commonplace. In comparison, the well-observed incidence of hemophilia is one in 5,000 male births, or one in 10,000 persons. Half of persons with hemophilia are severely affected, whereas less than five percent of persons with VWD are severely affected. The preponderance of mild forms in VWD contributes to uncertainty about prevalence.

The pathogenesis of VWD and the nature of the relationship between FVIII and VWF were not elucidated until the late 20th century. As comprehension improved, terminology changed. Standard terms, approved by the International Society on Thrombosis and Hemostasis, are used in this publication (Table 1). Older terms are in quotations.

History

In 1926, Dr. Erik von Willebrand of Helsinki published, in Finnish, his astute observations on a large family with a bleeding disorder from one of the Åland islands in the gulf between Sweden and Finland. He described all the clinical symptoms mentioned above. He surmised that inheritance was dominant but that the most severely-affected members of the inbred family were homozygotes. The only clearly abnormal laboratory tests, among the few available at the time, were the bleeding time (BT) and a capillary fragility test (the latter performed by inflating a blood-pressure cuff around the upper arm, to a level between systolic and diastolic pressure for a few minutes and observing the forearm for petechiae.) The platelet count was normal but there appeared to be minor morphologic changes in the platelets (not subsequently substantiated). The whole blood clotting time (which is prolonged in severe hemophilia) was normal. Dr. von Willebrand concluded that the disorder was due to “a disturbed function of the thrombocytes and a general lesion of the capillary walls” and called it “pseudohemophilia.

In 1928, Dr. George Minot of Boston described five patients from two families with similar symptoms and prolonged bleeding times. Dr. Minot’s name was associated with the disorder in the USA.
At first, VWD was believed to be a disorder of small blood vessels, at least in part. The disorder was called “vascular hemophilia” or “familial capillary fragility”. Telangiectasia was described in some patients but may have been co- incidental. Gastrointestinal angiodysplasia was described more often and was suspected to be co- incidental; excessive bleeding from such lesions in VWD was not surprising. Reports of capillary tortuosity, seen in the nail-beds, have been published from time to time. Recent studies have shown that VWF is necessary for normal angiogenesis. VWD may turn out to include a disorder of blood vessels after all.

The saga of gradual enlightenment about the nature of VWD is described below because classic scientific papers, still often cited, contain allusions and terms that can be understood only in the historical context.

In the 1950’s, with development of an assay for FVIII, hemophilia A could be defined clearly. Its inheritance was obviously sex-linked. All affected males in a given family had approximately the same (low) level of FVIII. In VWD, FVIII levels also could be low, but levels varied among affected members of a given family. The presence of low FVIII levels in an autosomal disorder was mystifying.

Transfusion of plasma became commonplace in the 1950’s. When plasma was transfused into patients with severe VWD, it corrected the FVIII level and the BT performed by the method of Duke (piercing the ear-lobe). After plasma transfusion, the FVIII level remained elevated much longer in VWD than in hemophilia A. As an experiment, plasma from patients with severe hemophilia A was transfused into patients with severe VWD. Duke BTs were corrected. The FVIII level rose slowly over a few hours, peaked a day after transfusion and returned gradually, over a few days, to the baseline level (Figure 1). Plasma from patients with severe VWD was transfused into patients with severe hemophilia A with no benefit. These “cross-transfusion experiments” suggested that a plasma factor, deficient in VWD, could be supplied by hemophilic plasma as well as by normal plasma. (Such experiments would not be performed today, for fear of transmitting infection.)

![Figure 1. Cross-transfusion.](image)

*FVIII levels in a patient with severe VWD after infusion of plasma from a patient with hemophilia A (data combined from several experiments).*
Throughout the 1960’s the relationship between FVIII and the plasma factor deficient in VWD (not yet named VWF) remained puzzling.

In the 1970’s, an antibiotic, ristocetin, was withdrawn from clinical use because it sometimes caused thrombocytopenia. It proved useful as a reagent. Australians showed that ristocetin stimulated the aggregation of platelets suspended in plasma (platelet-rich plasma, PRP) from normal persons or persons with hemophilia A but not from persons with (moderate to severe) VWD. If platelet-poor plasma from a normal person or a person with severe hemophilia A was added to PRP from a person with severe VWD, aggregation could then be induced by ristocetin, proving that a plasma substance (rather than a platelet substance) was missing in VWD. A quantitative test for VWF, the ristocetin cofactor test (VWF:RCo), was designed.

Immunological tests also helped clarify matters. When rabbits were injected with semi-purified FVIII (which, unbeknownst to the investigators, also contained VWF), the animals developed antibodies that recognized a substance in the plasma of all persons with hemophilia A. That substance was called “factor VIII related antigen”, “FVIIIIR:Ag” (now known to be VWF:Ag). Further studies showed that the semi-purified FVIII was composed of two entities, FVIII and VWF, which could dissociate.

In the 1980’s, VWF was found to be a large multimer composed of repeating subunits. Immunoelectrophoresis displayed multimers according to size and allowed the first attempts to segregate VWD types, which were consistent within families. The first official classification, in 1984, based on multimer patterns, used Roman numerals (Table 1). In “type I”, multimers of all sizes were present but in decreased concentration. In “type II”, the largest multimers (high molecular weight, HMW) were absent. In “type IIA”, no HMW multimers appeared after DDAVP stimulation, and, there was little or no aggregation of PRP with ristocetin. In “type IIB”, HMW multimers did appear after DDAVP stimulation but had a short half-life, and, patient PRP aggregated with levels of ristocetin too low to aggregate normal PRP, that is, the patient PRP hyper-aggregated. In “type III” (severe) VWD, all multimers were absent and PRP did not aggregate with ristocetin.
In 1985, the VWF gene was cloned. The gene and plasma protein were sequenced. Structure-function relationships could be studied. In the 1990’s, other functional tests were designed. VWF binding to collagen (VWF:CB) was described and became a popular test in Australia, with limited success elsewhere. In France, a few patients with low FVIII, first thought to have mild hemophilia A, were shown to have defective binding of VWF to FVIII, (VWF:FVIIIIB). This condition was named type 2N, (N for Normandy, origin of the index family).

In 1994, a slightly revised classification of VWD types used Arabic numerals (Table 2). Types 1 and 3 were believed to be quantitative defects; type 2 was qualitative. At that time, all VWD was postulated to be related to abnormalities of the VWF gene, but subsequent searches for gene abnormalities in type 1 were often disappointing. Except for defining four subtypes of type 2, further change in nomenclature has been resisted.

<table>
<thead>
<tr>
<th>Type of test</th>
<th>Official</th>
<th>Old or informal</th>
<th>Official</th>
<th>Old or informal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunological (total amount, functional or not)</td>
<td>FVIII:Ag</td>
<td>FVIII:CAg</td>
<td>VWF:Ag</td>
<td>FVIII-R:Ag, AHF:Ag</td>
</tr>
<tr>
<td>Functional (functional assay)</td>
<td>FVIII</td>
<td>FVIII:C, AHF, AHG (anti-hemophilic factor or globulin)</td>
<td>VWF:RCo (ristocetin cofactor) VWF:CB (collagen binding) VWF:FVIIIIB (factor VIII binding)</td>
<td>FVIII-R:RCo and others VWF:CBA</td>
</tr>
</tbody>
</table>

Table 1. Official abbreviated terms for factor VIII and von Willebrand factor, as designated by the International Society on Thrombosis and Hemostasis (ISTH), compared to old or informal terms.
Table 2. Laboratory patterns in VWD, according to type as defined by International Society on Thrombosis and Hemostasis. Table partly adapted from Ng et al, Blood 2015, 125: 2029-37.

<table>
<thead>
<tr>
<th></th>
<th>FVIII</th>
<th>VWF:Ag</th>
<th>VWF:RCo</th>
<th>VWF:RCo/Ag</th>
<th>Multimers</th>
<th>Other tests</th>
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<tr>
<td>QUANTITATIVE DISORDERS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1</td>
<td>Low</td>
<td>Similarly low</td>
<td>Similarly low</td>
<td>About 1, or &gt; 0.6</td>
<td>Normal, decreased amount</td>
<td>(Type 1 C diagnosed with elevated propeptide)</td>
</tr>
<tr>
<td>Type 3</td>
<td>Very low</td>
<td>Undetectable</td>
<td>Undetectable</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QUALITATIVE DISORDERS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 2N</td>
<td>Low</td>
<td>N to low, higher than FVIII</td>
<td>N to low, higher than FVIII</td>
<td>More than 0.6</td>
<td>Normal</td>
<td>Decreased VWF:FVIII binding capacity</td>
</tr>
<tr>
<td>Type 2M</td>
<td>N to low</td>
<td>Low</td>
<td>Lower than VWF:Ag</td>
<td>Less than 0.6</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>Type 2A</td>
<td>N to low</td>
<td>Low</td>
<td>Lower than VWF:Ag</td>
<td>Less than 0.6</td>
<td>HMW loss</td>
<td></td>
</tr>
<tr>
<td>Type 2B</td>
<td>N to low</td>
<td>Low</td>
<td>Lower than VWF:Ag</td>
<td>Less than 0.6</td>
<td>HMW loss</td>
<td>RIPA excessive; thrombocytopenia possible</td>
</tr>
<tr>
<td>Pseudo-VWD</td>
<td>N to low</td>
<td>Low</td>
<td>Lower than VWF:Ag</td>
<td>Less than 0.6</td>
<td>HMW loss</td>
<td>RIPA excessive; thrombocytopenia possible</td>
</tr>
<tr>
<td>(platelet-type)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
VWF gene and molecule

The VWF gene on chromosome 12 is very large, consisting of 178 kilobases with 52 exons. (A partial VWF pseudogene is located on chromosome 22.)

VWF is synthesized in endothelial cells and megakaryocytes. A signal peptide facilitates entry into the endoplasmic reticulum. A propeptide mediates alignment of VWF dimers into N-linked multimeric forms. In the endoplasmic reticulum, subunits join end-to-end by disulfide bonds to form dimers. In the Golgi apparatus, dimers link by additional disulfide bonds to form multimers ranging in molecular weight up to 10,000 kilodaltons and more. Mature VWF is stored in alpha granules in megakaryocytes and platelets, and in Weibel-Palade bodies in endothelial cells. VWF is secreted from endothelial cells into the plasma.

After secretion, the propeptide separates and circulates briefly. In the plasma, VWF multimers are subject to cleavage by a metallo-protease, ADAMTS-13 (which is deficient in thrombotic thrombocytopenic purpura.)

VWF is essential for the adhesion of platelets to the subendothelium at high fluid shear rates. VWF binds to subendothelial collagen and then to platelets at their glycoprotein Ib (GPIb) site. High molecular weight (HMW) VWF multimers bind to GPIB far better than do smaller ones. (Binding of VWF to FVIII is not dependent on multimer size.) After platelets activate, another binding site, glycoprotein IIb/IIIa (GPIIb/IIIa), becomes available to VWF. Binding of VWF at GPIIb/IIIa helps bridge the adherence of platelets to each other. VWF circulates as a coiled multimers. Upon stimulation, it uncoils into a long string, exposing many GPIb binding sites.
**Normal variation in VWF levels**

Levels of VWF and of FVIII are increased by environmental influences, as follows: (1) with adrenalin release as in strenuous exercise or stress, (2) with inflammatory conditions, (3) with severe liver disease, (4) with hyperthyroidism and (5) with high levels of estrogen and progesterone as in pregnancy.

Levels also are related to blood group and race (tables 3 and 4). Levels are higher with blood groups A and B compared to blood group O. (Group O is associated with a lower level of protein glycosylation; VWF is less glycosylated in group O persons. Levels of the protease ADAMTS-13 are higher and the clearance of VWF is faster in group O persons.) Levels of VWF are higher in persons of black African descent than in Caucasians. The lower limit of the normal range for African-Americans of non-O blood groups is almost twice as high as that of Caucasian-Americans of group O. Various normal inherited variations in the VWF gene (single nucleotide polymorphisms) also influence levels of VWF, in part by affecting susceptibility to proteolysis.

Diagnosis is difficult in persons with mildly-deficient or borderline test results. Factor levels vary slightly day-to-day within a given person; a value may be slightly in the abnormal range one day and within the normal range another day. Repeated tests, however, are of little help in diagnosis.

Given the difficult diagnosis, in an ideal world, the patient suspected of having VWD should go in person to a highly-reliable laboratory where (1) the PFA-100 is available, or a BT by an expert and (2) RIPA is available. Diagnosis of VWD type often depends on the ratio of VWF:RCo to VWF:Ag and therefore (3) all analyses should be done on one sample of blood (to avoid variation in levels due to more stress on one occasion than another) and (4) all analyses should be calibrated against the same normal pooled plasma standard (these vary slightly; diagnoses based on ratios are exceptionally vulnerable to such variation).

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**Table 3. ABO blood groups and VWF:Ag in normal persons (redrawn from Gill JC et alia, Blood 1987; 69:1691). Note the marked differences in the lower limit of normal, if defined as the mean minus two SD.**

<table>
<thead>
<tr>
<th>Blood group</th>
<th>n</th>
<th>VWF:Ag mean, %</th>
<th>Range, ± 2 SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>456</td>
<td>74.6</td>
<td>35.6-157.0</td>
</tr>
<tr>
<td>A</td>
<td>340</td>
<td>105.9</td>
<td>48.0-233.9</td>
</tr>
<tr>
<td>B</td>
<td>196</td>
<td>116.9</td>
<td>56.8-241.0</td>
</tr>
<tr>
<td>AB</td>
<td>109</td>
<td>123.3</td>
<td>63.8-238.2</td>
</tr>
</tbody>
</table>

**Table 4. Relationship of ABO blood groups and race to mean factor levels in 123 normal women (redrawn from Miller et alia, J Thromb Haemost 2003; 1:2191.)**

<table>
<thead>
<tr>
<th>Race &amp; ABO Blood group</th>
<th>VWF:Ag</th>
<th>VWF:RCo</th>
<th>FVIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>White, O</td>
<td>84</td>
<td>80</td>
<td>90</td>
</tr>
<tr>
<td>Black, O</td>
<td>104</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>White, non-O</td>
<td>113</td>
<td>110</td>
<td>109</td>
</tr>
<tr>
<td>Black, non-O</td>
<td>140</td>
<td>112</td>
<td>132</td>
</tr>
</tbody>
</table>
Types of VWD

VWD is very heterogeneous. Attempts to categorize cases into types helps comprehension but the lines are sometimes blurred. In general, types 1 and 3 are defects in the quantity of VWF but in type 2 there are various qualitative defects. Roughly, 80% or more of affected persons have type 1, about 15% have type 2, and 5% or less type 3.

Type 1 VWD

Type 1 VWD is the most common type. It is characterized by a mild to moderate mostly-quantitative deficiency of VWF affecting multimers of all sizes. Plasma VWF is functionally fairly normal. Levels of VWF:Ag and VWF:RCo are decreased to a similar extent. The proportion of HMW multimers is not significantly decreased.

The 1994 ISTH goal of associating all VWD diagnoses with demonstrable mutations has not been met for Type 1. In research studies, gene mutations were more likely to be identified in type 1 patients with definitely low levels of VWF and less likely to be found in those with borderline VWF levels. Mutations, typically missense, are in scattered sites and are dominant, some highly-penetrant, others with variable expression, perhaps depending on interaction with other genes. One person with a given VWF genotype may be asymptomatic and have laboratory tests in the normal range while another has mild to moderate symptoms with one or more laboratory tests in the abnormal range. Some highly-penetrant type 1 gene mutations are dominant-negative, that is, a mutant VWF retained intracellularly impedes the secretion of the normal VWF made according to the other, normal allele. A few mutations are “null”, that is, they code for no production of VWF. In homozygotes or double heterozygotes, null mutations cause type 3, or severe, VWD. Conversely, only a small minority of null mutation heterozygotes have a VWD phenotype.

Some apparent mild type 1 VWD may not be due to mutations in the VWF gene. Persons with excessive bleeding and with mildly-abnormal or borderline test results may have other genetic determinants, such as blood group O, that depress the VWF level. Perhaps a slightly low level of VWF is a risk factor for excessive bleeding, especially if accompanied by other defects, such as minor platelet function abnormalities. The family history may be positive, for such conditions may run in families.

Some observers refer to “Type 1C” VWD, “C” for “clearance”, a subgroup characterized by a significant increase in the clearance of VWF from the plasma, a higher than normal response to DDAVP (with a short half-life), and an elevated plasma level of the VWF propeptide versus mature VWF (a ratio that marks increased clearance).

Since 2000, three large multi-center studies (in Canada, the UK and Europe) have attempted to better define type 1. See the annotated references for details.

Diagnosis of type 1 VWD is easy when the personal and family histories of excessive bleeding are clear-cut and when levels of FVIII, VWF:Ag, VWF:RCo and VWF:CB (collagen binding, where available) are similar and are definitely below the normal range. The BT sometimes is prolonged. Ristocetin induced platelet aggregation, RIPA, usually is normal or nearly so.
Figure 4. A family with type 1 VWD in at least four generations. The inheritance is autosomal dominant, highly penetrant. Levels of FVIII, VWF:Ag and VWF:RCo are roughly similar within each affected person.

Figure 5. A family with type 2A VWD, autosomal dominant, male-to-male transmission. Note that VWF:RCo is distinctly more deficient than VWF:Ag.
Type 2A VWD

Typical type 2A VWD is caused by dominant loss-of-function mutations, usually missense and usually in the A2 domain, or sometimes the A1 domain (see table 5). Penetrance is high. With some mutations, multimer formation is impeded and no HMW multimers ever are formed. With other mutations, HMW multimers form but are vulnerable to proteolysis and don’t last long enough to circulate.

Absence of HMW multimers greatly reduces VWF binding to platelet GPIb. The depressed function is reflected in a decidedly low ristocetin cofactor (VWF:RCo) and collagen binding (VWF:CB), a reduced to absent ristocetin-induced platelet aggregation (RIPA) and a prolonged BT. The total amount of VWF, measured as VWF:Ag (which reflects large and small multimers), is only mildly depressed, and, therefore, is higher than VWF:RCo or VWF:CB. Low molecular weight VWF multimers bind FVIII normally so the level of FVIII is similar to that of VWF:Ag. The diagnosis of VWD is usually obvious in these patients.

Type 2B

Type 2B VWD is caused by dominant gain-of-function mutations, usually missense, in the A1 domain of the VWF gene. Penetrance is high. The mutant VWF has increased affinity for platelet GPIb. HMW multimers are constantly removed from circulation. There may be mild thrombocytopenia.

Laboratory findings reflect the functional deficiency caused by the loss of HMW multimers: a decidedly-low VWF:RCo and VWF:CB and a prolonged BT but only a mild deficiency of VWF:Ag and FVIII. In strong contrast to type 2A, RIPA is excessive, that is, platelet aggregation is robust at low concentrations of ristocetin. The diagnosis of VWD is usually obvious in these patients but differentiation of type 2A and 2B requires RIPA or mutation analysis.

Type 2 M

In the uncommon type 2M VWD (“M” for “multimer”), VWF binding to platelet GPIb is defective, as reflected by a decidedly-low level of VWF:RCo, however, multimers of all sizes are present. The level of VWF:CB is only mildly deficient compared to the more-deficient VWF:RCo. That difference can be used to predict the subtype. The responsible mutations are in the A1 domain. Certain patients, originally described in Vicenza, Italy, circulate multimers larger than any seen in normal plasma. Mutations responsible for the Vicenza phenotype are in the D3 domain.

Type 2N

Type 2N (“N” for “Normandy”, home of the first patient described) is caused by recessive mutations in the D’ to D3 domains which inactivate the VWF binding site to FVIII. Patients with homozygous or doubly heterozygous mutations have low FVIII levels, similar to those of patients with mild or moderate hemophilia A. Levels of VWF:Ag and VWF:RCo can be normal, low-normal, or mildly depressed. Heterozygotes have normal levels of FVIII but sensitive tests may demonstrate decreased VWF-FVIII binding.
Table 5. Effect of mutations in various domains of VWF gene and type of VWD resulting from those mutations, from Fressinaud E, Mazurier C, Meyer D. International J Haematol 2002, 75:9-18

<table>
<thead>
<tr>
<th>Domain</th>
<th>Predominant type</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>D2</td>
<td>rare 2A</td>
<td>Decreased large multimers, increased proportion of protomers, decreased satellite bands, recessive inheritance. Mutation presumably interferes with VWF processing &amp; multimer assembly.</td>
</tr>
<tr>
<td>D’</td>
<td>2N</td>
<td>FVIII binding affected. Some mutations also induce a quantitative reduction in VWF and a decrease in HMW forms. Recessive inheritance. Heterozygotes have intermediate FVIII binding.</td>
</tr>
<tr>
<td>D3</td>
<td>(2N if close to D’), 2M Vicenza</td>
<td>Ultra-high molecular weight VWF multimers present, recognized only on low-resolution gels with very large pore size. (With other gels, multimer pattern looks like type 1.) The pathogenesis is not understood.</td>
</tr>
<tr>
<td>A1</td>
<td>2B</td>
<td>Gain-of-function mutations with increased binding of HMW VWF to platelet GPIb receptors. The defect can be subtle and a few patients are misclassified as 2A.</td>
</tr>
<tr>
<td>A1</td>
<td>2M, 2A</td>
<td>Other mutations cause decreased affinity of VWF for platelet GPIb, with (2A) or without (2M) loss of HMW VWF.</td>
</tr>
<tr>
<td>A2</td>
<td>2A</td>
<td>Loss of HMW multimers. Group 1 = defective intracellular transport of VWF leading to impaired secretion of VWF multimers in plasma and platelets. Group 2 = increased sensitivity of VWF to proteolysis in plasma.</td>
</tr>
<tr>
<td>A3</td>
<td>New, no name</td>
<td>Mild disorder with decreased binding to collagen.</td>
</tr>
<tr>
<td>CK</td>
<td>2A</td>
<td>Absence of large multimers in plasma and platelets, abnormal internal structure of multimers seen with high resolution gels. Rare, dominant.</td>
</tr>
</tbody>
</table>
**Type 3 VWD**

Patients with homozygous or doubly heterozygous null mutations or deletions make little or no VWF. Such mutations have been found throughout the gene. VWF:Ag and VWF:RCo are below the level of detection. Bleeding into joints may occur, but is not nearly as frequent as in severe hemophilia A, probably because a small amount of FVIII does circulate in most patients. Homozygosity for large deletions in the gene is associated with vulnerability to allo-antibody formation (anti-VWF, inhibitor of VWF). Most heterozygotes are asymptomatic but laboratory test results may be at the lower end of the normal range.

An occasional patient, previously diagnosed as having severe hemophilia A, will, on testing of VWF levels, be found to have type 3 VWD instead. He is likely to respond better to concentrates containing VWF as well as FVIII.

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**Pseudo-VWD (Platelet-type VWD)**

A gain-of-function mutation in the platelet gene for GPIb causes increased affinity of that ligand for HMW VWF multimers. The dominantly-inherited highly-penetrant platelet disorder has a phenotype similar to type 2B VWD. Laboratory differentiation from type 2B may be difficult and may depend on gene analysis. Patients typically have prolonged BTs, borderline to normal levels of FVIII and VWF:Ag, low levels of VWF:RCo, absent HMW multimers, enhanced RIPA at low concentrations of ristocetin and thrombocytopenia. Bleeding is treated with platelet transfusions.

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*Figure 6. A family with type 3 VWD in the youngest member, who has severe bleeding symptoms but no joint bleeding (note that his FVIII level of 5% is like that of a moderate-mild hemophilia A patient). The mother and maternal grandfather have mild bleeding symptoms and may have type 1 VWD.*
Acquired VWD

A VWD syndrome, presumed to be due to auto-antibodies, may appear in patients with hypothyroidism, autoimmune disorders, lymphoma, macroglobulinemia or other similar conditions. Auto-antibodies to VWF are much more difficult to document in the laboratory than inhibitors in hemophilia.

VWD also can be acquired by mechanical damage. Turbulent blood flow, as with shear stress at abnormal heart valves, clears the largest multimers. Plasma levels of the VWF propeptide are typically higher than normal. A majority of patients with serious valvular disease can be shown to have the syndrome if laboratory studies are done, but only a few are symptomatic. Repair or replacement of the valve cures the VWD.

VWD and atherosclerosis

Pigs with type 3 VWD have less atherosclerosis than is seen in normal pigs, even if they are fed high cholesterol diets from an early age. Humans with moderate to severe VWD are not protected from atherosclerosis, but have a lower prevalence of arterial thrombotic events than normal persons.

Figure 7. Part of an extensive kindred with pseudo-VWD. The two brothers in the youngest generation might not be diagnosed if only their VWF levels were measured, and if platelet counts were not done. Both men had markedly excessive bleeding from trauma. In affected family members, hemostasis could be achieved only with infusions of fresh platelets.
**Symptoms**

The most common symptoms in children are repeated nosebleeds and excessive bruising. The frequency of nosebleeds diminishes in adulthood. Excessive bleeding after trauma or surgical operations, especially after dental extractions and other procedures in the mouth and nose, occur at any age and may be the presenting problem. Menorrhagia is the predominant symptom in females and may be incapacitating. Onset of menorrhagia at menarche is typical. Ovarian cysts are more prevalent than in unaffected women. The prevalence of gastrointestinal bleeding increases with age, and may reflect the increasing prevalence of gastrointestinal angiodysplasia with age. Bleeding into joints or muscles may occur in type 3 VWD. A standardized questionnaire, a “bleeding assessment tool” or “BAT”, creating a “bleeding score”, has been established but is more popular and useful in research than in clinical practice.

Menorrhagia may be overlooked because the patient may not realize that her menses are atypical and because quantitation of menstrual blood loss is difficult. A “pictorial blood loss assessment chart”, “PBAC”, helps women score their own menstrual blood loss according to the number of sanitary tampons and napkins used and their degree of saturation with blood.

In pregnancy, levels of FVIII and VWF rise to double the baseline values by term. In type 1 VWD, levels of functionally-normal VWF may reach the normal range. In type 2 VWD, levels of VWF rise, but the VWF is still dysfunctional and peripartum hemorrhage is more likely than in type 1 VWD. Women with type 1 VWD who still have subnormal levels of VWF at term, and women with types 2 and 3 VWD are usually given FVIII-VWF concentrates. Alternatively, responsive women with type 1 VWD may be given DDAVP after delivery. Levels of FVIII and VWF fall to near baseline within a week of delivery, thus, late post-partum hemorrhages may occur.

**Laboratory Tests**

Bleeding times (BT) are insensitive and non-specific. They can be prolonged by a variety of conditions. BTs are typically prolonged in patients whose VWD is easily diagnosed with other tests. The “Ivy” BT is more sensitive than the older “Duke” BT.

Automatic platelet function analyzers, predominantly the PFA-100® made by Dade International, have been used in recent years. Anti-coagulated fresh whole blood passes at a high sheer-rate through a capillary tube and through a collagen-coated membrane, in the presence of ADP or epinephrine, to an aperture. Formation of a platelet plug, closing the aperture, is signaled by a closure-time, CT. Closure-times are more sensitive (that is, abnormal in a higher percentage of patients with VWD) than are bleeding times but are just as non-specific (that is, prolonged by many different conditions).

The two tests using ristocetin may be confused (Figure 8). There are three reagents in the ristocetin co-factor test, as follows: (1) plasma diluted to various degrees, (2) a standard amount of normal platelets and (3) a standard amount of ristocetin. The concentration of ristocetin is high, relative to the amount of VWF in any test-tube, providing maximum stimulation. Aggregation of platelets can be observed visually or measured by optical density. The more dilute the plasma, the longer the time to aggregation.
Figure 8. Tests using ristocetin.

In the ristocetin cofactor test (top row of five tubes), the level of ristocetin is constant but dilutions of plasma vary.

In ristocetin induced platelet aggregation (bottom row of two tubes), platelet-rich plasma is constant but dilutions of ristocetin vary.
Figure 9. Reference graph for ristocetin cofactor. The agglutination times of normal platelets, suspended in normal plasma and stimulated with ristocetin, are plotted against the dilution of the plasma.

In this example, plasma from a VWD patient diluted to 50% (1:2) has an agglutination time similar to normal plasma diluted to 15%; 15 x 2 = 30% VWF:RCo.

His plasma diluted to 25% (1:4) has an agglutination time similar to normal plasma diluted to 8%; 8 x 4 = 32% VWF:RCo.
Figure 10. Ristocetin induced platelet aggregation. Tracings of RIPA at two final concentrations of ristocetin, 1.2 and 0.6 mg/ml, from my collection, are pictured above. The patient with type 1 VWD had baseline FVIII, VWF:Ag and VWF:RCo levels of 10%. Platelet-rich plasma from the patient with type 2B VWD also aggregated at a final ristocetin concentration of 0.3 mg/ml. RIPA in pseudo-VWD is indistinguishable from that seen in type 2B.
A standard curve, plotted with normal reference plasma, relates dilution to aggregation time (Figure 5). The aggregation times of a patient's plasma are compared to the standard curve to quantitate von Willebrand factor activity as VWF:RCo. Levels of VWF:RCo are low in all types of VWD except 2N. The test can be performed on plasma that has been frozen and thawed. The normal platelets are formalin-fixed and either frozen or freeze-dried.

One must be alert to the few normal black persons who have a polymorphism in the VWF gene that causes a spurious low VWF:RCo result. This polymorphism does not affect the VWF:CB.

In ristocetin induced platelet aggregation, (RIPA), there are two reagents, as follows: (1) fresh platelet-rich plasma (PRP) and (2) ristocetin at two different concentrations. The PRP is obtained by slow centrifugation of fresh anticoagulated blood. The higher of the two ristocetin concentrations is one that always stimulates aggregation in normal PRP, the lower concentration is one that never stimulates aggregation in normal PRP. Use of the second, lower concentration of ristocetin reveals any tendency to over-respond that would be obscured with a maximal response to the higher concentration. RIPA is usually normal or only slightly diminished in type 1 VWD. It is absent or diminished in type 2A but is robust at the lower concentration of ristocetin in type 2B VWD and in pseudo-VWD (Figure 10). The use of RIPA is limited because it must be performed on fresh blood.

Some laboratories substitute other methods of measuring VWF function. One test uses monoclonal antibodies to platelet GPIb, fixed to latex particles. Other tests have been developed (see references). The VWF:RCo is still regarded as the gold-standard.

ELISA tests measuring VWF binding to collagen, VWF:CB, reflect another VWF function. VWF:CB is low in all the VWD types in which VWF:RCo is low, except for type 2M, in which it is clearly not as deficient as VWF:RCo. That differential can be used for subtype diagnosis, especially if multimer analysis is not available.

An ELISA test for VWF binding to FVIII, VWF:FVIIIb, is available in a few laboratories. In type 2N VWD, binding is virtually absent. Heterozygotes have intermediate levels. Persons with other types of VWD have normal levels.

Tests for VWF:Ag, using antibodies to VWF, are easily obtained. The test measures the total amount of the protein, not its function, thus, in type 2 VWD, VWF:Ag levels typically are higher than those of VWF:RCo or VWF:CB.

Quantitation of VWF:Ag propeptide (PP) is available in a few specialized laboratories.

Electrophoresis of VWF in gels can show differential migration of multimers of different sizes. Antibodies to VWF mark the multimers, along with a label that creates a visible record. Gels with low resolution suffice to distinguish type 2 VWD from type 1, that is, the presence or absence of HMW multimers. Gels with high resolution normally show one or two satellite bands on either side of the predominant band formed by each size of multimer. The most elite, research-level multimer laboratories often find minor abnormalities not distinguished in routine labs.
**CHOICE OF DIAGNOSTIC TESTS**

Some clinicians like to use a bleeding assessment tool (BAT), a questionnaire to evaluate the severity of the bleeding history. In suspected VWD, initial laboratory tests usually consist of VWF:Ag, VWF:RCo, FVIII and a platelet count. A VWF:CB may be done where available (in a few families, this is the sole abnormal test!) If levels of VWF are extremely low, then type 3 is diagnosed. If levels of FVIII or VWF are less than 30% (or 40%, depending on the criteria chosen) and roughly equal (the ratio of VWF:RCo/ VWF:Ag is more than 0.6), then type 1 is suspected. (If levels of FVIII and VWF are between 30 and 50%, the clinician may use the term “possible VWD.”) Some clinicians might then measure the VWF propeptide, proportionately elevated in type 1C, which will predict rapid clearance of VWF.

If the level of FVIII is disproportionately lower than that of VWF:Ag, then type 2N is suspected and a test for VWF:FVIIIB obtained. If the level of VWF:RCo is disproportionately lower than VWF:Ag (ratio 0.6 or lower) then type 2A, 2B or 2M are suspected. Differentiation of these may require multimer analysis and RIPA, if available. If the VWF:CB test is available, and higher than VWF:RCo, then type 2M is suspected. Mutation analysis of part of the VWF gene is recommended if some sort of type 2 VWD, or pseudo-VWD, is suspected.

Given the difficult diagnosis, in an ideal world, the patient suspected of having VWD should go in person to a highly-reliable laboratory where (1) RIPA is available. Diagnosis of VWD type often depends on the ratio of VWF:RCo to VWF:Ag and therefore (2) all analyses should be done on one sample of blood (to avoid variation in levels due to more stress on one occasion than another) and (3) all analyses should be calibrated against the same normal pooled plasma standard (these vary slightly; diagnoses based on ratios are exceptionally vulnerable to such variation). In addition, (4) PFA-100 should be available in preference to a BT.

**TREATMENT**

**Local care**

Prolonged local pressure on small wounds is useful in any bleeding disorder. If nasal packing is used for nosebleeds, the material should be easy to remove without disturbing fragile clots. Gauze lightly impregnated with a lubricant (e.g. Vaseline®) is popular. Dr. Marion Koerper of San Francisco uses a frozen piece of firm animal fat (“salt pork”) which is easy to trim to size and to slide out intact. Cauterization is not advised because burned areas eventually slough, often with renewed bleeding.

Local hemostatic agents are sometimes used for nosebleeds or the sockets of extracted teeth. Products include Surgicel® (an adherent but absorbable cellulose) or Gel-foam® (absorbable gelatin sponge), sometimes fortified with thrombin powder. Fibrin glue has been used in tooth sockets.

**Avoid anti-platelet agents**

In normal persons, ingestion of aspirin doubles the Ivy BT. Aspirin may greatly prolong the BT and accentuate clinical bleeding in VWD. Most anti-pyretic and analgesic agents, such as acetaminophen (paracetamol) and most non-steroidal anti-inflammatory drugs have no such effect.
**Estrogen-progesterone**

Estrogen-progesterone pills, even in the low doses used for contraception, decrease endometrial proliferation and may suffice to control mild menorrhagia. High-dose pills may be tried if the low dose is insufficient. The pills may be given continuously over many months to reduce the frequency of menses. Intravenous estrogen may be used to stop an episode of severe menorrhagia, e.g., Premarin® 25 mg every four hours for up to six doses. Vaginal rings or intrauterine devices providing slow release of estrogen and progesterone or progesterone alone are well-tolerated in mature women.

**Anti-fibrinolytic agents**

Epsilon-amino-caproic-acid (EACA, Amicar®) and tranexamic acid (Cyclokapron®) both inhibit plasminogen activator, thus preventing formation of plasmin, which lyses fibrin. They are often highly effective for mouth or nose bleeding, for menorrhagia and for dental extractions. They sometimes are adequate as sole therapy, or, they may be combined with DDAVP or clotting factor concentrate. Oral doses for adults are ten grams/day of EACA or 3900 mg/day of tranexamic acid, both in divided doses. One of these drugs might be taken each day for five days to help control menorrhagia (see references). Intravenous formulations also are available, e.g. for use before surgical procedures.

**DDAVP**

DDAVP (deamino-8-D-arginine vasopressin, desmopressin), is a synthetic analog of the natural hormone vasopressin, without vasopressin’s pressor effect but with a much stronger water-retaining effect. It releases FVIII, VWF and plasminogen activator from storage sites. It also directly causes mild activation of platelets and enhancement of their adhesion to injured endothelium.

In normal persons, within an hour of a standard intravenous dose of 0.3 micrograms (ug) /kg, or a standard adult intranasal dose of 300 ug (150 ug sprayed into each nostril), levels of plasma FVIII rise about three-fold and levels of VWF about two-fold. The intranasal form is known as Stimate®. Doses of DDAVP also can be given subcutaneously. If doses are closely-spaced, e.g. every 24 hours, responses to the second and later doses may be less than that to the first dose (tachyphylaxis), as would be expected if stores of FVIII and VWF must be replenished (Figure 12). Low-dose DDAVP nosedrops used for diabetes insipidis are inadequate in VWD.

Some persons with types 1 and 2 VWD have good responses to DDAVP but some have poor responses (related to genotype). Some patients, e.g. type 1C, have a robust initial response but rapid half-life; the initial response may be adequate to halt bleeding. The level of response on different occasions tends to be similar within a given person and kindred. A test dose often is administered given after a VWD diagnosis to define the patient’s response.

DDAVP is used to stop acute bleeding in responsive patients with type 1 VWD and in some with type 2A or 2M VWD (Table 6). The bleeding time may be corrected temporarily in type 1 VWD and improved in type 2A or 2M. DDAVP suffices to prevent excessive bleeding from minor surgical procedures in most patients with type 1 VWD who are responsive to the drug.
Figure 11. A good response to DDAVP, in a standard intravenous dose of 0.3 micrograms/kg. The patient had type 1 VWD with baseline FVIII and VWF:RCo levels of 10%.

Figure 12. Tachyphylaxis. Anticipated response of a patient with type 1 VWD given DDAVP on each of three consecutive days, showing a lower response on the second and third day than on the first (based on data averaged from several subjects.)
DDAVP is not advocated for use in type 3 VWD. It may suffice to control acute bleeding in type 2A VWD. Its use in type 2B VWD is undergoing re-evaluation. When DDAVP is given to a patient with type 2B VWD, fresh HMW multimers are released and promptly aggregate circulating platelets, causing temporary mild to moderate thrombocytopenia. The platelets do not appear to be either activated or destroyed. Platelet aggregates may simply disaggregate. The platelet count improves within an hour. Some clinicians fear that the temporary thrombocytopenia may exaggerate acute bleeding, others have used DDAVP in type 2B without problems.

Adverse events may occur with the use of DDAVP, as follows:

1. The release of plasminogen activator may cause a "paradoxical" increase in bleeding. Some clinicians give an antifibrinolytic agent with all doses of DDAVP. (That is my own policy.)

2. Water intoxication may ensue, especially in patients with unrestricted access to oral fluids or in patients receiving hypotonic intravenous fluids. Fluid restriction is advised.

3. Deep vein thrombosis or myocardial infarct have been reported, especially in

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**Table 6.** In type 2A VWD, DDAVP releases more of the dysfunctional VWF, but it may be adequate to halt minor bleeding. This patient had an actively oozing tooth socket of two days’ duration but the bleeding stopped, permanently, immediately after this infusion of DDAVP.

<table>
<thead>
<tr>
<th>Time</th>
<th>BT min</th>
<th>FVIII %</th>
<th>VWF:Ag %</th>
<th>VWF:RCO %</th>
<th>Bleeding %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-infusion</td>
<td>30+</td>
<td>32</td>
<td>22</td>
<td>8</td>
<td>Moderate, tooth socket, two days</td>
</tr>
<tr>
<td>30 min. post</td>
<td>30+</td>
<td>50</td>
<td>37</td>
<td>16</td>
<td>Stopped permanently</td>
</tr>
</tbody>
</table>

**Table 7.** In type 2B VWD, DDAVP releases more of the HMW VWF avid for platelet binding, so there may be a temporary but marked decrease in platelet count. The patient described above received a test dose of DDAVP; he was not bleeding at the time.

<table>
<thead>
<tr>
<th>Time</th>
<th>BT min</th>
<th>FVIII %</th>
<th>VWF:Ag %</th>
<th>VWF:RCO %</th>
<th>Platelets/cu mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-infusion</td>
<td>20+</td>
<td>21</td>
<td>25</td>
<td>10</td>
<td>176,500</td>
</tr>
<tr>
<td>30 min. post</td>
<td>20+</td>
<td>105</td>
<td>100</td>
<td>72</td>
<td>26,000</td>
</tr>
<tr>
<td>2.5 hrs. post</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>123,500</td>
</tr>
</tbody>
</table>
older patients with risk factors for such
events. In the 1980’s, when HIV transmis-
sion was feared, patients without bleeding
disorders were sometimes given DDAVP to
reduce blood loss in major surgery. Two
meta-analyses of large clinical trials in such
patients yielded opposite opinions on the
contribution of DDAVP to thrombotic compli-
cations. Many clinicians remain uneasy
about giving DDAVP to older patients.

Plasma and cryoprecipitate

Fresh-frozen plasma or cryoprecipi-
tate were used in the past, and may be used
today in less-affluent countries, to treat pa-
tients with VWD who do not have adequate
responses to DDAVP. Because of the risk of
transmitting viral infections with plasma
products, viral-inactivated concentrates are
now preferred, where available.

Plasma or cryoprecipitate immedi-
ately elevates the patient’s levels of FVIII
and VWF. The Ivy BT cannot always be cor-
corrected even with large doses of cryoprecipi-
tate. (The BT may depend on the VWF level
within platelets). Hemostasis is excellent de-
spite the recalcitrant BT, even in surgical
procedures.

The half-life of the infused VWF:RCo,
which depends on the survival of large mul-
timers, is about 10 hours. The level of FVIII
continues to rise over several hours or a day
because the exogenous VWF (including the
longer-lasting small multimers as well as the
short-lived large ones) transports the pa-
tient’s endogenously-produced FVIII.
In the cryoprecipitate era, before VWF:RCo could be measured, I treated patients with severe VWD with enough cryoprecipitate preoperatively to achieve a 100% plasma FVIII level and used half that dose every 12 hours afterwards. Patients with milder VWD were prepared similarly for the operating room and were given cryoprecipitate after surgery at intervals of 24 hours or longer. The dosages used provided excellent hemostasis and probably were overly generous. Before cryoprecipitate was available at all, fresh-frozen plasma served well, despite provision of lower amounts of VWF. I relate this experience because clinical trials on the use of concentrates for surgical operations in VWD describe higher dosages, probably much higher than needed.

VWF-FVIII concentrates

A variety of plasma-derived concentrates of FVIII with VWF have been used to treat VWD patients inadequately responsive to DDAVP. The concentrates approved by the FDA for use for VWD in the USA are the following: (1) Humate-P® (spelled Haemate-P and Hemate-P in some other countries), made in Germany by CSL Behring. It has more than two international units (IU) of VWF:RCo per IU of FVIII and retains HMW multimers well. (2) Alphanate®, made in the USA by Grifols. It contains about equal amounts of VWF:RCo and FVIII (it is identical to Fanhdi®, made in Spain). (3) Wilate®, made by Octapharma in Europe from plasma collected in the USA. It has approximately equal amounts of VWF:RCo and FVIII. Biostate® is made in Australia from Australian plasma and is available there. Other FVIII-VWF concentrates may be made and available in other countries.

Early publications on the use of FVIII-VWF products reported dosage in terms of FVIII international units (IU) before the development of international standards for VWF measurement. Nowadays dosage is quoted in VWF:RCo IU.

No dose-finding studies have been conducted. Information about dosage comes from case reports and from large series in which doses used in various types of VWD were averaged.

The concentrates named above were reported to be highly effective in controlling bleeding episodes and preventing excessive bleeding in surgery. In recent clinical trials of VWD treatment for surgery, the chosen dosages kept the postoperative patient’s plasma level of VWF:RCo close to 100% for several days. Patients’ plasma levels of FVIII often rose higher than did levels of VWF:RCo. The total post-infusion FVIII level consists of infused exogenous FVIII plus endogenously-released FVIII. Plasma levels of FVIII sometimes greatly exceeded the upper limit of normal. A slightly-increased incidence of post-operative deep vein thrombosis in VWD, compared to hemophilia A, has been blamed on such very high FVIII levels.

Figures 14 and 15 combine data from multiple sources to illustrate the effect of the relative concentrations of FVIII and VWF:RCo on the dosage needed and the peak levels of FVIII that may be reached.

Dosage can be calculated using the patient’s weight and the concentrate’s content of VWF:RCo as stated on the label. Infusion of each IU of VWF:RCo/kg is expected to raise the patient’s plasma level of VWF:RCo by about two percentage points, e.g., an infusion of 50 VWF:RCo IU/kg should raise the plasma level of VWF:RCo from zero to about 100%.
Figure 14. Surgical dosage, concentrate with more FVIII than VWF. Hypothetical mean daily factor levels in a patient with severe VWD treated for surgery with an initial dose (40 IU of VWF:RCo/kg and 80 IU of FVIII/kg) and subsequently with doses half that much every 12 hours, with the goal of maintaining the plasma VWF:RCo around 100%, using a concentrate with 0.5 IU of VWF:RCo per IU of FVIII. After several days of use, plasma levels of FVIII may exceed normal range.

Figure 15. Surgical dosage, concentrate with more VWF than FVIII. Hypothetical mean daily factor levels in a patient with severe VWD treated for surgery with an initial dose (40 IU of VWF:RCo/kg and 20 IU of FVIII/kg) and subsequently with half that much every 12 hours, with the goal of maintaining the plasma VWF:RCo level around 100%, using a concentrate with 2 IU of VWF:RCo per IU of FVIII.
Patients with type 1 VWD who do not respond adequately to DDAVP may be treated with concentrate. To raise the level of VWF:RCo from a baseline of, e.g., 20% to a desired level of 50%, an increment of 30 percentage points, the patient should receive about 15 IU VWF:RCo/kg. In patients with type 2 VWD, that is, with abnormal VWF, I consider the level of VWF:RCo to be zero when calculating dosage.

One dose of concentrate, sufficient to raise the plasma level of VWF:RCo to 50%, is generous treatment for most acute hemorrhages, deep injections, and simple dental extractions. For surgical operations, generous dosage might include an initial dose that raises the level of VWF:RCo to 100% and subsequent doses, half the loading dose, every 12 hours to maintain the VWF:RCo level above 50%. Post-operative treatment might continue for one or two days for minor procedures. I treat patients having major surgery for 10 days, as for hemophilia care.

Patients with bleeding or surgical operations in the nose, mouth or throat, and patients having dental extractions, who are receiving concentrates typically also receive anti-fibrinolytic drugs.

Concentrates containing FVIII alone, including those derived from plasma using monoclonal-antibody affinity chromatography, and all recombinant FVIII concentrates, may be helpful to control hemorrhages in patients with VWD when no concentrate containing VWF is available. Such concentrates are sub-optimal therapy for VWD. The rare patients with type 3 VWD and inhibitors to VWF may be treated with concentrates of FVIII alone with some benefit.

**Concentrates of VWF**

LFB in France produces a plasma-derived VWF concentrate, Wilfactin®, with very low levels of FVIII. For acute bleeding, a dose of 40 VWF:RCo IU/kg, or slightly more, was effective. For surgery, patients with severe or moderately-severe VWD received a first dose some 12-24 hours before the operation (to allow the patient’s endogenous FVIII to be expressed and circulated) and a second dose immediately pre-operatively. For acute bleeding or preparation for emergency surgery, a FVIII concentrate could be given in addition to the VWF concentrate.

A new, recombinant VWF concentrate, Vonvendi® (Baxalta), does not contain FVIII. Large multimers are well-preserved because no ADAMTS-13 is present. The half-life of the infused VWF:RCo is a little longer than with plasma-derived products. For acute bleeding or preparation for emergency surgery, a FVIII concentrate could be given in addition to the VWF concentrate. For prophylaxis, scheduled surgical operations or post-operative care, the VWF concentrate alone should suffice, given that the VWD patient can make his own FVIII.

**Platelets**

Fresh platelets are the mainstay of therapy for pseudo-VWD. Platelets (which contain VWF) have sometimes been useful, in addition to FVIII-VWF concentrate, in VWD patients with persistent bleeding, in particular from the gastrointestinal tract.
Treatment of Menorrhagia

Control of menorrhagia may require multiple agents. Estrogen-progesterone pills or devices and anti-fibrinolytic agents may be tried first, if insufficient, then DDAVP nasal spray, then concentrates. Some women require daily prophylactic concentrate starting at the onset of the menstrual period. In women who have completed childbearing, endometrial ablation or hysterectomy is considered.

Dental restorations and extractions

Minor mucosal injuries during dental restorations are controlled relatively easily with pressure, local agents, anti-fibrinolytic agents or, for major procedures, DDAVP or concentrate. Tooth sockets may be packed with various local hemostatic agents. Injections for regional block anesthesia can cause dangerous hemorrhages. Vascular bundles accompany nerves. Accidental piercing of a small vessel at the angle of the jaw can cause a hematoma that can dissect down the neck and press on the trachea. Preparation with DDAVP or concentrate is advisable, as for a minor surgical procedure.

Prophylaxis

Regardless of the type of VWD, patients with frequent, severe bleeding such as hemarthroses, gastrointestinal bleeding or heavy epistaxis may benefit from regular prophylactic infusions of a concentrate containing VWF. Some patients get along on weekly infusions, some on twice weekly, and some, especially with gastrointestinal bleeding, require more frequent doses. Doses of 30-50 IU VWF:RCo/kg have been used. If dosing must be given more than once or twice a week, a VWF concentrate, rather than a FVIII-VWF concentrate, should be used to avoid the development of too high a level of FVIII.
Comprehensive review


Reviews, primarily biochemistry


The preproVWF precursor contains five kinds of structural domains in the order D1-D2-D'-D3-A1-A2=A3-D4-B1-B2-B3-C1-C2-CK. In the endoplasmic reticulum, proVWF dimerizes through disulfide bonds between C-terminal cystine knot (CK) domains. ProVWF dimers are transported to the Golgi, where a protease cleaves after the propeptide (D1-D2) and additional disulfide bonds form between D3 domains. Large VWF multimers are compressed into tubular structures in Weibel-Palade bodies.


VWF is stored as ultra-large VWF multimers in Weibel-Palade bodies. On secretion from endothelial cells, a proportion of these UL-VWF multimers remain anchored to the activated endothelium. The multimers unravel, bind platelets, and wave in the direction of the flow. These VWF “strings” can be extraordinarily long, and make available many platelet binding and ADAMTS13 cleavage sites.


Reviews, including clinical


A patient with a history of mucocutaneous bleeding can be diagnosed with VWD if the
VWF activity is <30%. Patients with such a history and a VWF activity level of 30-50% have “low VWF”, a risk factor. Testing for binding to both GP Ib and collagen is recommended, but activity tests using monoclonal antibodies against the VWF GP Ib are not yet sufficiently reliable. If the ratio of VWF:RCo/VWF:Ag or VWF:CB.VWF:AG is <0.6, then type 2 is suspected and RIPA and multimer analysis are justified. A trial of DDAVP is recommended for all but type 2B and type 3 patients and those with known atherosclerosis. For surgical operations, factor VIII levels should be raised to 100% and maintained above 50% in the postoperative period. VWF:RCo should be maintained above 50% in the perioperative period.


Bleeding scores, the APTT, the BT and the PFA-100 are not universally sensitive and specific. If VWD is suspected, specific tests must be done: VWF:RCo, VWF:Ag, FVIII. If the ratio VWF:RCo/VWF:Ag is < 0.6, then multimer tests and RIPA are justified. If FVIII is abnormally low compared to VWF:Ag, then test for VWF:FVIIIb. If VWF:RCo/VWF:Ag ratio is >0.6, then type 1 is diagnosed, and if the VWF:PP (propeptide)/VWF:Ag level is elevated, then type 1C can be diagnosed. (Excellent algorithms and charts.)

Terminology


The terminology “Types I, IIA, IIB and III” was based on analysis of VWF multimer size in plasma, with a decrease in multimers of all sizes in “type I”, absence of large multimers in “type II” and absence of all multimers in “type III”. DDAVP provokes emergence of multimers of all sizes in “type I”, of further small multimers but no large ones in “type IIA”, of small and larger multimers (the latter with a short half-life) in “type IIB”, but no response in “type III”.

Zimmerman TS, Ruggeri ZM. Von Willebrand’s disease. Clinics in Haematology 1983. 12:175-199. (Similar to above, outstanding figures.)


The 1984 classification based solely on multimer patterns did not accommodate types described since that time, in particular, patients in whom multimer patterns were normal but VWF was clearly abnormal. The new classification declared that all VWD is caused by mutations at the VWF gene locus. (This requirement could not be met for type 1.) The previously-named “platelet-type VWD” was to be called “pseudo-VWD” and acquired deficiency of VWF due to development of autoantibodies was to be called “acquired von Willebrand syndrome”. The many rare cases that had been described in the previous decade were gathered into major types and Arabic numerals used, instead of the former Roman numerals, to distinguish the new classification. In general, types 1 and 3 were described as quantitative and type 2 as qualitative.

VWF cDNA The nucleotide sequence should be numbered from the A of the initiator ATG site as the +1 position. Genomic DNA should be prefixed with a “g” and also numbered from this position. Amino acid (aa) numbering should be from the initiator methionine at the +1 position with sequential numbering of amino acids throughout VWF.


VWD is caused by inherited defects in the concentration, structure or function of VWF. Type 1 is partial quantitative deficiency, type 3 is virtually complete deficiency. Type 2, qualitative defects, includes 2A with deficiency of HMW multimers, 2B with increased affinity for platelet glycoprotein Ib, 2M with defective platelet adhesion despite a relatively normal multimer distribution and type 2N with decreased affinity for FVIII. “Some VWF gene mutations... have complex effects and give rise to mixed VWD phenotypes.” Types 1 and 2A “encompass several pathophysiologic mechanisms.” The heterogeneity of VWD was recognized, as was the difficulty of finding gene mutations especially in type 1.


Substitutes for the ristocetin cofactor test have been developed, leading to a need for a standard nomenclature for them, (but are not yet in common parlance, so were not discussed in the text.) The official names are as follows in the next column:

<table>
<thead>
<tr>
<th>ABBREVIATION</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>VWF:RCo</td>
<td>Ristocetin cofactor activity, all assays that use platelets and ristocetin</td>
</tr>
<tr>
<td>VWF:GP1bR</td>
<td>All assays that are based on the ristocetin-induced binding of VWF to a recombinant wild-type GPIb fragment</td>
</tr>
<tr>
<td>VWF:GP1bM</td>
<td>All assays that are based on the spontaneous binding of VWF to a gain-of-function mutant GPIb fragment</td>
</tr>
<tr>
<td>VWF:Ab</td>
<td>All assays that are based on the binding of a monoclonal antibody (mAb) to a VWF A1 domain epitope</td>
</tr>
</tbody>
</table>

History, early descriptions


This is an English translation of the original article, which appeared in Finnish in 1926.


Symptomatic persons in two families had prolonged bleeding times and epistaxis, bruising and prolonged bleeding from small cuts. Male to male transmission was noted in one family, ruling out sex-linked inheritance. Platelet counts and whole blood clotting times were normal. Dr. Minot’s name was associated with the condition in the USA in early years.
hemorrhages with Cohn fraction I-O prepared from normal plasma. On one occasion she was transfused with fraction I-0 made from the pooled plasma of patients with severe hemophilia A. Two hours later, her FVIII level had risen to 12% and her Duke BT was normal. At 24 hours post-infusion her FVIII level was 19%. On another occasion, she received fraction I-0 made from the plasma of patients with VWD and her FVIII level did not rise.


In France, patients with hemophilia A served as donors of fresh blood or plasma for eight different recipients with VWD. The Duke BTs were corrected in all seven recipients in whom they had been prolonged. Levels of FVIII rose gradually, peaking in the low-normal to normal range (44-81%) at 4-24 hours post-transfusion.

In the USA, a girl with VWD, with a prolonged Duke BT and plasma FVIII levels of 10-15% was transfused with normal plasma and, on another occasion, with plasma from a person with severe hemophilia A. FVIII levels rose within one hour after transfusion of plasma from either source and peaked by four hours. Dr. Lewis suggested that the girl was able to synthesize her own FVIII when infused with a substance present in hemophilic plasma.

Discoveries made using ristocetin


Ristocetin was an antibiotic then recently
withdrawn from clinical use because of a high incidence of thrombocytopenia. These Australian investigators noted that in vitro, ristocetin induced aggregation in normal platelet-rich plasma but not in platelet-rich plasma from two of three patients with VWD. They recognized the potential utility of ristocetin aggregation in diagnosing VWD. That is, they did a ristocetin-induced-platelet aggregation test, RIPA, usually deficient in type 2A and 3 VWD but often normal in type 1 VWD.

Ristocetin-induced platelet aggregation was absent or markedly decreased in the platelet-rich plasma of ten patients with VWD. The defect could be corrected by adding normal plasma or plasma from a person with hemophilia A to the VWD patient’s platelet-rich plasma. They concluded that patients with VWD were deficient in a plasma factor (VWF) necessary for normal platelet function. They suggested that VWF was “associated with” the FVIII molecule.


Addition of normal platelet-poor plasma to the platelet-rich plasma of VWD patients with absent RIPA restored the ability to respond to ristocetin, suggesting that a factor present in plasma (rather than a platelet factor) was deficient in VWD.


A semi-purified FVIII concentrate, made from normal plasma, corrected the defective platelet adhesiveness to glass seen in VWD. The concentrate was believed, therefore, to contain VWF, the factor missing in VWD. The semi-purified FVIII was injected into rabbits who made an antibody to something in it. The antibody inhibited the ristocetin-induced aggregation of normal platelet-rich plasma. They concluded that a von Willebrand factor existed in plasma and was needed to support ristocetin aggregation.


The investigators described an assay for VWF activity in which washed normal platelets were combined with various dilutions of normal plasma and aggregation was stimulated by ristocetin (the ristocetin cofactor test). In normal subjects, a highly significant correlation was found between levels of FVIII and of VWF (measured as ristocetin cofactor), which suggested that the two factors were closely associated.

Discoveries using immunologic tests


An antibody (inhibitor) to FVIII, arising in a human, was neutralized by a substance in the plasma of only two of 54 patients with moderate to severe hemophilia A, indicating that most patients with severe hemophilia A do not make a FVIII molecule. The two patients who were exceptions were presumed to make a non-functional FVIII molecule. (Contrast to the results in the next paper.)
Zimmerman TS, Ratnoff OD, Powell AE. Immuno-
logic differentiation of classic hemophilia (factor VIII deficiency) and von Willebrand’s dis-

“Factor VIII” (believed, at the time, to be highly-purified) was injected into rabbits who made antibodies to it (in reality, to VWF). Using this antibody, a substance described as “AHF -like antigen” (AHF = anti-hemophilic factor = factor VIII) was detected in the plasma of 22 patients with hemophilia A in normal levels but was decreased in the plasma of eleven patients with VWD.


"Factor VIII antigen" (later termed VWF:Ag), identified by rabbit antibodies, was located within cultured endothelial cells and in the culture medium around these cells. Radio-labeled amino acids were incorporated into the “factor VIII antigen”. By tracing the label, the authors showed that endothelial cells synthesize and release a protein that shares antigen present on normal human “factor VIII” (later recognized as the FVIII-VWF complex). No FVIII coagulant activity, how-
ever, was found in the culture medium.

Montgomery RR, Zimmerman TS. Von Wille-

A second VWF antigen (later recog-
ized as VWF propeptide) was detected by an-
tibodies raised in rabbits.

Fay PJ, Kawai Y, Wagner DD, Ginsburg D et alia. Propolypeptide of von Willebrand factor circ-
ulates in blood and is identical to von Willebrand antigen II. Science 1986: 232:995-998.

Von Willebrand antigen II was recog-
nized as the 100-kilodalton pro-peptide “that is first cleaved from pro-VWF during intracel-
ular processing and then released into plasma.”

FVIII and VWF are two entities

McLester WD, Graham JB. Nature 1964; 201:1040-1042

Several hypotheses about the rela-
tionship between FVIII and VWF were enter-
tained. VWF might be an activator or a regula-
tor of FVIII production, or, FVIII and VWF might be produced separately and combine. (Hypotheses were discussed in other early publica-
tions but this is the first I can find that con-
tained a correct guess.)

Owen WG, Wagner RH. Antihemophilic factor: Separation of a active fragment following disso-
ociation by salts or detergents. Thromb Diath Haemorrh 1972, 27:502-515

Using salts and detergents, a low-
molecular-weight FVIII component with co-
agulant activity could be dissociated from a very high molecular weight component. They proposed that the HMW component was a carrier for the smaller, active component.

Zimmerman TS, Edgington TS: Factor VIII co-
agulant activity and factor VIII- like antigen: inde-

Multiple antibodies against “factor VIII” preparations were raised in rabbits, some affecting VWF:Ag and some affecting FVIII coagulant activity. An anti-FVIII inhibitor arising in a human also was obtained. The antibodies were fixed to agarose beads. FVIII or VWF:Ag was each adsorbed by its specific antibody, leaving the other entity in the su-
pernatant, showing they were separable.

“Factor VIII” was separated into two components, a low-molecular weight component with FVIII coagulant activity and a higher molecular weight component with “VWF activity” as measured by ristocetin aggregation tests. It was not yet clear whether these two components were “separate molecules or… subunits of a complex macromolecule.” They believed that “the intact complex is a polymer that includes repeating subunits.”

**Crossed-immuno-electrophoresis**

Before VWF was electrophoresed in multimers, this test aided discoveries.


Three patients had long BTs and absent RIPA. Their VWF:Ag, present at normal or only mildly-reduced levels, migrated unusually rapidly to the anode on crossed immuno-electrophoresis (i.e. electrophoresis was carried out in one direction and then in the perpendicular direction.) (If all multimers are small, they travel more rapidly than a mixture of small and large multimers.) These patients had type 2 VWD. Several other reports followed.

**Demonstration of Multimers**


“Factor VIII” (the FVIII-VWF complex) in aggregated form, with a MW over a million, migrates in large pore polyacrylamide gels. “Dialysis against buffers of decreasing ionic strength results in the appearance of faster moving bands of increasing intensity. This result suggests that the factor VIII preparation contains a homologous series of oligomers the distribution of which depends on the ion strength of the medium.”


Purified porcine VWF was analyzed by sodium dodecyl sulfate (SDS)-agarose electrophoresis. Multiple forms were seen in a series of increasing molecular weight, from about $1.1 \times 10^6$ to $2.1 \times 10^7$. The difference between one series and another was about $1.5-1.9 \times 10^6$ daltons, “indicating that members of the series were polymers of 6-mers to 8-mers of the $2.3 \times 10^5$ dalton subunit.”


A series of multimeric forms of VWF:Ag was discovered using SDS agarose electrophoresis. Their size was estimated as MW $0.85$ to $12 \times 10^6$.

Ruggeri ZM, Zimmerman TS. Variant von Willebrand’s disease. Characterization of two subtypes by analysis of multimeric composition of factor VIII/von Willebrand factor in plasma and
The multimeric composition of factor VIII/VWF in plasma and platelet lysates were studied using SDS agarose electrophoresis followed by incubation with a radio-iodine-labeled antibody to VWF and exposure to X-ray film to make a visible record. In normal plasma, ten distinct multimer bands were seen, ranging in apparent molecular weight from 0.86 to 9.9 x 10^6. A normal multimeric pattern was seen in patients with type 1 VWD. In plasma from type 2A VWD, only the five smaller multimers were present in significant amounts, with traces of the 6th and 7th sometimes seen. Large multimers also were absent in platelet lysates from type 2A VWD. In plasma from type 2B VWD, intermediate-sized multimers were more evident than in type 2A, with the 7th and 8th multimers easily detected. The multimer pattern in type 2B platelets was the same as in normal platelets, that is, all normal multimer sizes were represented.

In Italy, electrophoresis of VWF:Ag in platelets showed that large multimers of VWF were present in type 1 and type 2B platelets but not in type 2A platelets. After DDAVP, multimers of all sizes emerged into the plasma of patients with type 1 VWD, no large multimers emerged in type 2A VWD, and some fairly-large multimers emerged in type 2B VWD but rapidly disappeared.

In England, the same classification of VWD as described above was demonstrated with multimer patterns and RIPA in 116 patients from 47 families. (This paper is recommended for its outstanding figures.)

Other early observations on VWF


Citrated whole blood was circulated over everted rabbit aortas denuded of epithelium. Adhesion of the platelets of four patients with VWD to the subendothelium was definitely subnormal; that of a fifth patient with mild VWD was only mildly subnormal.


VWF appeared to stabilize FVIII in vitro and perhaps also in vivo. They proposed that although FVIII and VWF were apparently made separately, FVIII was stabilized by VWF.


Large oligomers of the FVIII-VWF complex bound preferentially, with high affinity, to low capacity sites on platelets.


A monoclonal antibody against platelet GPIb prevented ristocetin-induced binding of VWF but had no effect on thrombin- or ADP-epinephrine-induced binding of VWF. A monoclonal antibody against platelet GPIIb/IIIa had no effect on ristocetin-induced binding of VWF but blocked thrombin- or ADP-epinephrine-induced binding of VWF. Thus, platelets have two binding sites for VWF.


VWF bound to polymeric forms of a variety of collagen types. Larger VWF multimers bound better than smaller ones.


When type I fibrillar collagen was incubated with plasma or with purified FVIII/VWF, the largest multimers of VWF were selectively adsorbed. The authors suggested that HMW VWF acts as a subendothelial collagen-platelet bridge.


VWF:Ag synthesis was studied in cultured endothelial cells. A subunit was produced intracellularly as a glycoprotein of MW 240,000, and cleaved to a size of 225,000 on secretion into the culture medium.


Within the endothelial cell, subunits of pro-VWF formed pro-VWF dimers. Dimers underwent post-translational modification including glycosylation and sulfation. Dimers linked with disulfide bonds to form multimers. The pro-sequence was cleaved.


The VWF protein was sequenced.


Pre-treatment of normal vessel walls with antibodies to VWF inhibited adherence of platelets. Inhibition was shear-rate dependent, being significant at high shear-rates. VWF present in the vessel wall appeared to contribute appreciably to platelet adherence.


A fluid shear stress of 180 dyn/cm² was applied to platelets in citrated plasma or blood in a viscometer. Platelets aggregated if large VWF multimers were present, without the stimulus of ristocetin or other agents. Very large VWF multimers produced by endothelial cells were more effective than the largest VWF multimers in plasma in supporting shear-induced aggregation. Aggregation was
inhibited by monoclonal antibodies to platelet GPIb or GPIIb-IIIa.


Observations on VWF in the new century

VWF multimers of different sizes were prepared from normal cryoprecipitate and were radio-labeled. Ristocetin-dependent binding to GPIb and thrombin-dependent binding to GP IIb/IIIa were better with large multimers than with smaller ones.


VWF was purified from normal plasma and from plasma of patients with type 2A and 2B VWD. Binding affinity of type 2A VWF to platelet receptor GPIb was greatly decreased, compared to normal, and that of type 2B VWF was increased. Binding of both 2A and 2B VWF to GPIb-IIa was decreased.


The precise sequence of interactions among VWF and platelet receptors GPIb and GPIIbIIIa were studied with a video microscopy system. "Adhesion at high shear rates was the result of the adsorption of large VWF multimers onto collagen and the binding of platelet GPIb to the insolubulized VWF. Aggregation occurred subsequently and required the binding of ligands including VWF...to GPIIb-IIIa."


In thrombotic thrombocytopenic purpura (TTP), proteolysis of VWF is decreased and the plasma contains unusually large multimeric forms of VWF. Congenital TTP (a recessive condition) had appeared in seven families. ADAMTS 13, a metalloproteinase, was identified and its deficiency shown to be responsible for TTP. A genetic locus was detected on chromosome 9q34. Twelve different gene mutations were identified. Heterozygotes had intermediate levels of deficiency.

Arya M, Anvari B, Romo GM, et alia. Ultralarge multimers of von Willebrand factor form spontaneously high-strength bonds with the platelet glycoprotein Ib-IX complex: studies using optical
Ultra-large VWF multimers bound to GPIb sites spontaneously (i.e. without ristocetin) and the strength of the bond was greater than normal. The A1 domains from these multimers were isolated and also bound to GP1b sites with greater than normal strength. A1 domains may be more exposed in very large multimers than in smaller ones. The conformational state of VWF multimers may be critical to function. The platelet binding site known simply as GPIb is called GP Ib-IX or GP Ib-IX-V in this and some other papers.


Exposure to shear stress causes VWF to unfold, increasing its capacity to support platelet aggregation and enhancing its susceptibility to cleavage by ADAMTS13. Abnormally high levels of shear stress across a stenotic aortic valve or in small vessels in hemolytic-uremic disease promote cleavage, with loss of the largest multimers.


FVIII gene expression (mRNA) was analyzed quantitatively in various tissues. FVIII levels were high in hepatic sinusoidal endothelial cells and Kupffer cells (but not in hepatocytes) and also in renal glomeruli and tubular epithelial cells. VWF was predominantly located in the endothelium of larger vessels.


Levels of FVIII and VWF:Ag normally are concordant. The level of FVIII relative to VWF:Ag is increased if VWF synthesis is reduced. An increased level of FVIII relative to VWF:Ag is found in persons bearing one null allele (heterozygotes for type 3 VWD) and also in persons with the subgroup of type 2A VWD with impaired intracellular transport of VWF and decreased secretion of VWF. FVIII and VWF:Ag are concordant in type 2A VWD with normal synthesis and secretion but increased extracellular proteolysis of VWF. Heterozygotes with one allele for 2N VWD and one normal allele have concordant levels of FVIII and VWF. A decreased level of FVIII relative to VWF is found in type 2N homozygotes or compound heterozygotes with one allele for 2N and one null allele. The authors propose that VWF contains an excess of unoccupied FVIII binding sites. All FVIII synthesized is normally bound by VWF. A reduction of VWF, e.g. to 50% as by a null allele, still provides enough carrying capacity for most of the FVIII produced. When the VWF:Ag level is very low, some unbound FVIII circulates.


Under shear forces, the VWF A1 domain can assume the role of the A3 domain to trigger platelet recruitment to collagen fibers. The primary binding site for collagen has been thought to be the A3 domain. Collagen binding may be sequential: first the A3 domain, then the A1 domain. If the A1 site can substitute for the A3, that helps explain the dearth of VWD cases secondary to poor collagen binding alone.

Previous studies showed that expression of FVIII in VWF-producing cells results in co-localized storage of VWF and FVIII in endothelial cell Weibel-Palade bodies or in alpha granules of megakaryocytes. In Type 2N VWD, both FVIII and VWF are released concomitantly after DDAVP. This study showed that, despite the binding problems in type 2N, co-expressed FVIII can be targeted to the VWF-containing Weibel-Palade bodies. The full story of the marriage of FVIII and VWF has not yet been told.


Platelet VWF, stored in alpha granules, is enriched in HMW multimers. N-linked glycosylation is markedly reduced (50%) compared with plasma VWF. Because of the lesser glycosylation, platelet VWF resists ADAMTS13 proteolysis. Platelet activation at sites of injury releases high local concentrations of HMW multimers from platelet VWF that is more resistant to ADAMTS13 thus facilitating platelet plug formation.


BOECs from four type 1 patients, 4 type 2 patients and 9 controls were studied. VWF mRNA and protein levels and multimers were studied. Decreased mRNA levels were predictive of plasma VWF levels in type 1, confirming a defect in synthesis. However, BOECs from this group also showed defects in processing, storage and/or secretion of VWF. Levels of mRNA and protein were normal in BOECs from three of the type 2 patients including a 2A patient and two related 2M patients, supporting the “current notion of defective extracellular processing of VWF”. An unrelated type 2M patient had decreased VWF synthesis and storage, indicating a complex cellular defect.

The VWF gene described


The gene for VWF was located on chromosome 12. Using cDNA, 618 basepairs were sequenced. (Four other groups defined the gene in the same year.)


Domains of the VWF gene are defined and their repetitions enumerated.


The VWF gene is about 178 kilobases in length. The signal peptide and propeptide are encoded by 17 exons in about 80 kb, and the mature subunit and 3' noncoding regions by 35 exons in the remaining kb. Given its size and number of exons (52) the VWF gene is the most complex of the genes encoding hemostatic proteins. The entire nucleotide sequence is quoted.

Type 2 VWD variants were matched to missense mutations in specific areas of the VWF gene related to function. Mutations causing types 1 and 3 were not related to specific sites in the gene. Type 3 VWD usually was caused by nonsense, frameshift and deletion mutations, in homozygotes or double heterozygotes. Some clearly-dominant type 1 VWD appeared to be caused by dominant negative mutations.

Prevalence of VWD


This article is described in detail because it was influential; I believe its conclusions are mistaken. In Vicenza province, Italy, 1281 supposedly-normal children ages 11-14 were surveyed. Bleeding symptoms in the child, his siblings and his parents were determined by questionnaire. Plasma was tested for VWF:RCo. “Values of VWF obtained from children without a family history of hemorrhage were used for the definition of the normal range of VWF...Separate normal ranges were calculated for O and non-O subjects.” The normal range was defined as the area between the 2.5th and 97.5th percentiles. “Probable” VWD was diagnosed in subjects with a VWF level below that range and a family history of bleeding. “Definite” VWD was diagnosed when at least one other family member, from the side of the family with a bleeding history, had a VWF level below the normal range (using a normal range determined with normal adults). Ten children were classified as having VWD (6 definite, 4 probable) for an overall prevalence of 0.82%, or, if other statistical methods were used, as many as 14 children (prevalence 1.15%). (Details of these 14 children are given in a table; the diagnosis is unquestionable in one boy who had 8% VWF:RCo and whose family members had levels of 19-21% but is questionable in others whose levels of VWF:RCo range from 36 to 72%).


VWF:RCo was measured in 600 healthy children ages 2-18 years seen for routine examinations in the USA. Personal and family history of bleeding were determined by questionnaire. Diagnosis of VWD required a personal history of bleeding symptoms, a family history of at least one person with bleeding symptoms, and VWF:RCo below the 2.5th percentile of the distribution for the blood group (O, non-O). Eight subjects met the study criteria for VWD, of whom seven had blood group O. Of these, one had indistinguishable VWD with a VWF:RCo level of 10%. Of the remaining seven, one was tested on only one occasion and had 21% VWF:RCo. The other six were further tested and had borderline or low-normal levels of VWF:Ag and FVIII and normal multimer patterns. RIPA in three subjects was said to be diminished. A prevalence of VWD of 1.3% was claimed.


As of 1998, 761 patients with symptomatic VWD were registered in Sweden. Of these, 586 had type 1 VWD, 55 had 2A, 49 had 2B, 1 had 2M, none had 2N, 47 had type 3 and 23 were unclassified. Of the type 3 patients whose mutations had been analyzed, 21 were homozygotes and nine were compound heterozygotes. The prevalence of type 3 is about five per million population in Sweden. If mating were random, the number of heterozygotes for type 3 would be 27,000. (Their calculation showed that as many as 27,000 persons with one null allele existed in
Sweden. If they were symptomatic, there would be many more patients with “type 1” VWD than the 586 known to exist.)


Denmark had 5.2 million inhabitants of whom 250 were diagnosed with VWD, a prevalence of 4.8 per 100,000 inhabitants (or about 1 per 20,000). More females (#161) than males (#89) were diagnosed. They found 194 type 1 cases, 21 type 2A, 15 type 2B, 2 type 2N, 11 type 3 and 7 of unknown type.


A total of 1076 patients with VWD were registered in Finland, population about 5 million (roughly one in 5000 persons), including 695 type 1, 145 type 2 and 18 type 3.


In a survey of type 3 VWD, a VWF:Ag level of < 1% was verified in 154 subjects from 137 families. The prevalence of severe VWD was the highest in Sweden (3.23 per million people), Norway (2.5), and Finland (2.2). The overall prevalence for Europe plus Israel was 0.45 per million.


In Canada, patients attending family physicians were asked whether they had any problems with bleeding or bruising. Those with a sufficient bleeding score had tests for VWD. Nine of 30 persons had results compatible with VWD (7 type 1, 1 type 2B, 1 type 2M.) One control person with no history of bleeding had a low VWF level. The authors conclude that the prevalence of VWD is at least one in 1000. These subjects had not been seeking a diagnosis. A more restricted denominator might be persons seeking an explanation of excessive bleeding.

Variability of VWF, ABO effect


The least variation of FVIII and factor IX was seen within 74 sets of monozygotic twins, more within 84 like-sexed dizygotic twins, and the most between unrelated pairs. Higher levels of FVIII, VWF:Ag and factor IX were seen in older adults (ages 57-62) than in younger ones (ages 33-39). No gender differences were seen. ABO group was not correlated with factor IX level. Levels of FVIII and VWF:Ag were lowest in blood group O persons, higher in A-2 and highest in A-1 and B groups, as follows:

<table>
<thead>
<tr>
<th>Blood group</th>
<th>N</th>
<th>Mean VWF:Ag, %,*</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>60</td>
<td>65</td>
</tr>
<tr>
<td>A-2</td>
<td>20</td>
<td>87</td>
</tr>
<tr>
<td>A-1</td>
<td>68</td>
<td>100</td>
</tr>
<tr>
<td>B</td>
<td>15</td>
<td>103</td>
</tr>
<tr>
<td>A-1/B</td>
<td>4</td>
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</table>

* translated from natural logarithms.

**VWF:Ag and ABO blood group was measured in 1117 blood donors.** *(Table 3, page 6). Among 142 patients previously diagnosed with VWD, 77% of type 1 patients had blood group O vs. 31% of type 2 patients.). “There may be a subset of type I vWd patients with decreased concentrations of structurally normal vWF on the basis of blood group rather than specific inherited abnormalities of vWF production or release.”


Among 330 normal persons in Japan, those of blood group genotype AO or BO had slight decreases in VWF:Ag and VWF:RCo compared to persons with genotypes AA, AB or BB. Persons with genotype OO had much lower values for these factors, as follows:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>VWF:Ag, %, mean</th>
<th>VWF:RCo, %, mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>OO</td>
<td>68</td>
<td>80.9</td>
<td>82.2</td>
</tr>
<tr>
<td>AO</td>
<td>69</td>
<td>103.7</td>
<td>110.6</td>
</tr>
<tr>
<td>BO</td>
<td>52</td>
<td>100.5</td>
<td>107.3</td>
</tr>
<tr>
<td>AA</td>
<td>41</td>
<td>113.3</td>
<td>123.8</td>
</tr>
<tr>
<td>BB</td>
<td>31</td>
<td>114.5</td>
<td>125.3</td>
</tr>
<tr>
<td>AB</td>
<td>69</td>
<td>113.8</td>
<td>124.3</td>
</tr>
</tbody>
</table>


In a Dutch (white) population, a genome wide association study correlated VWF level with SNPs to a significant level in 6 of 8 genes studied: the ABO gene, the VWF gene and 6 others. The correlation of ABO genotype and VWF:Ag was as follows:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>VWF:Ag %, mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>OO</td>
<td>1536</td>
<td>108</td>
</tr>
<tr>
<td>A1-O</td>
<td>872</td>
<td>145</td>
</tr>
<tr>
<td>A2-O</td>
<td>300</td>
<td>114</td>
</tr>
<tr>
<td>BO</td>
<td>284</td>
<td>150</td>
</tr>
<tr>
<td>A1-A1</td>
<td>146</td>
<td>167</td>
</tr>
<tr>
<td>A1-A2</td>
<td>104</td>
<td>152</td>
</tr>
<tr>
<td>A1-B</td>
<td>83</td>
<td>158</td>
</tr>
<tr>
<td>A2-A2</td>
<td>17</td>
<td>134</td>
</tr>
<tr>
<td>A2-B</td>
<td>26</td>
<td>171</td>
</tr>
<tr>
<td>BB</td>
<td>8</td>
<td>178</td>
</tr>
</tbody>
</table>


**VWF:Ag levels and Secretor genotype were evaluated in 136 normal persons. When all ABO groups were considered together, significantly higher levels of VWF:Ag were found in persons homozygous for the Secretor allele than in heterozygotes.**

The rate of VWF proteolysis by ADAMTS13 was highest for blood group O, then B then A then AB. The VWF site for ADAMTS13 cleavage is flanked by glycosylation sites. Perhaps the presence of a blood group sugar on one or more sites may influence proteolysis, e.g. by steric hindrance or a charge effect.


The elimination half-life of VWF after DDAVP infusion was compared in normal subjects. The mean time was 10 hours in group O subjects (n=28) and was 20.9 hours in non-O subjects (n=19). Subjects with higher baseline levels of VWF had longer-surviving VWF.

Variability in VWF, racial differences


RIPA at the usual ristocetin level (1.1 mg/ml) in normal black subjects resulted in less than half the amplitude, on average, than seen in normal white subjects. The difference was decreased by increasing the ristocetin level to 1.5 mg/ml. Deficient RIPA in platelet-rich plasma from black subjects could not be corrected in vitro by adding plasma or platelets from white subjects who had normal aggregation.


Women with menorrhagia (70 black, 53 white) and age-matched controls (76 black, 47 white) were studied. Levels of VWF:Ag, VWF:RCo and FVIII all were significantly higher in group-non-O blacks than in group-non-O whites. In contrast, RIPA was significantly lower in blacks than in whites.


Further studies were conducted on the above normal female control subjects. Additional tests were VWF:CB and “VWF:MoAB”, the monoclonal Ab test measuring VWF activity. ABO blood group differences accounted for 19% of the total variance in VWF:Ag and race for 7% of the total variance. Mean results,%, were as follows:

<table>
<thead>
<tr>
<th></th>
<th>White O</th>
<th>Black O</th>
<th>White non-O</th>
<th>Black non-O</th>
</tr>
</thead>
<tbody>
<tr>
<td>VWF:Ag</td>
<td>84</td>
<td>104</td>
<td>113</td>
<td>140</td>
</tr>
<tr>
<td>VWF:RCo</td>
<td>80</td>
<td>80</td>
<td>110</td>
<td>112</td>
</tr>
<tr>
<td>VWF:CB</td>
<td>77</td>
<td>98</td>
<td>103</td>
<td>128</td>
</tr>
<tr>
<td>VWF:MoAB</td>
<td>75</td>
<td>83</td>
<td>98</td>
<td>132</td>
</tr>
<tr>
<td>FVIII</td>
<td>90</td>
<td>100</td>
<td>109</td>
<td>132</td>
</tr>
</tbody>
</table>


Levels of VWF:Ag and VWF:RCo were studied in a healthy control group of 59 African Americans (AA) and 113 Caucasians, with normal bleeding scores. AAs had a higher
mean level of VWF:Ag than did Caucasians, but levels of VWF:RCo were similar (and normal), thus, the VWF:RCo/VWF:Ag ratios were lower in the AAs. DNA sequencing revealed a trio of SNPs in exon 28 that appeared frequently in AAs but rarely in Caucasians. One of these SNPs, D1472H, was associated with decreased ratios in both racial groups. The few AAs who were homozygous for this SNP had the lowest ratios. Six AA subjects had RCo/Ag ratios less than six. The presence of the SNP did not affect VWF:CB, nor binding to a GpIb complex independent of ristocetin, i.e. it did not appear to affect VWF function. The authors warn that a normal person with this SNP and a borderline level VWF:Ag might have a VWF:RCo/VWF:Ag ratio that suggests type 2 M VWD.

Flood VH, Friedman KD...Montgomery RR et alia. No increase in bleeding identified in type 1 VWD subjects with D1472H sequence variation. Blood 2013; 121:3742-3744.

Following on the previous observation that the D1472H sequence variation was present in 63% of African American and 17% of healthy Caucasian controls but was not associated with an increased bleeding score, the authors looked at bleeding scores in their large collection of type 1 VWD subjects with VWF:Ag < 60%. The 36 subjects with the D1472H polymorphism had a mean bleeding score of 6.7 and a mean VWF:RCo of 29 %, whereas the 241 subjects without that polymorphism had a mean bleeding score of 5.4 (ns) but a higher mean VWF:RCo of 37 IU/dl (p=<.001). About 75% of subjects with and without the polymorphisms were of blood group O.

Additional sequence variations were found in 81% of the subjects with D1472H and 40% without that polymorphism. The presence of additional sequence variations in the D1472H group confounds interpretation of the results. Those subjects with D1472H and no other sequence variation had VWF levels similar to those observed in the group without D1472H. (Thus, the other sequence variations may affect the VWF:RCo level.)


The association of 30 nonsynonymous VWF variants with VWF and FVIII levels were assessed in 4468 African Americans. Nine of the 30 polymorphisms were significantly (p=<0.0017) associated with VWF:Ag and FVIII; five with lower levels and four with higher levels. The SNPs described account for about 3.3% of the overall phenotypic VWF variance whereas ABO blood group contributes about 15%.

Bellissimo DB, Christopherson PA... Montgomery RR et alia. VWF mutations and new sequence variations identified in healthy controls are more frequent in the African-American population. Blood 2012; 119: 2135-2140.

In VWF gene sequencing in 184 healthy controls (of whom 66 were African American, AA) without a history of abnormal bleeding, 21 new sequence variations were identified. Of these 13 occurred exclusively in AAs, and two were found in 10-15% of AAs, suggesting that they are polymorphisms. Fourteen sequence variations had previously been reported as mutations causing VWD type 1 and three of these putative mutations had frequencies of 15-18% in AAs. Ten AAs had a putative VWD type 2N mutation and one of these subjects was homozygous for it.

**Type 1 VWD**

Phenotypic variation within families. Blood 1979; 54:117–136. (The families in this often-quoted, detailed study had type 1 VWD.)

Two large families, long settled in North Carolina and known to have had VWD in seven generations, were studied for bleeding history, BT, FVIII, VWF:RCo and VWF:Ag. Inheritance appeared to be autosomal dominant with highly variable expression. Among 26 persons who had apparently transmitted the disorder (having had an ancestor and a descendant with VWD) only 13 had an abnormal laboratory test. The test most frequently abnormal was VWF:RCo; the test least frequently abnormal was the BT.


In 17 patients with mild to moderate type 1 VWD, there was an excellent correlation between BT and platelet VWF:RCo (r =.8) and a lesser correlation with platelet VWF:Ag (r =.5) All five patients with decreased platelet VWF:RCo had prolonged BTs whereas 10/12 patients with normal platelet VWF:RCo had normal BTs. Platelet vWF is an important determinant of the bleeding time.


Among of 246 patients previously diagnosed with type 1 VWD, only 144 had low VWF activity levels when adjusted for blood group. Those with either a notable personal or family history of bleeding were called ‘possible’ type 1 VWD (n=51) and those with both a personal and family history were called ‘definite’ type 1 VWD (n=88) Those 102 patients with blood group O, a personal and/or family history of bleeding and VWF levels between 35 and 50 % were an indeterminate group. Bleeding symptoms may depend on VWF levels regardless of ABO types. In a further paper, they reported that when better multimer analysis was available, some type 1 patients were reclassified as type 2.


A mutant VWF gene for highly penetrant, dominant, moderately severe type 1 VWD was transfected together with a normal gene bearing a marker. The mutant gene caused intracellular degradation of VWF with a decrease in VWF secretion. “Dominant type 1 VWD may be caused by heterodimerization of mutant and normal subunits in the endoplasmic reticulum followed by proteosomal degradation in the cytoplasm.”


This influential article was expanded in the next reference. This reference, however, includes a prevalence estimate of 23-113 symptomatic VWD cases per million population (i.e. up to 1:10,000) referred to specialized centers.


The distribution of VWF levels is very broad, low values seldom have a simple ge-
netic basis, and many bleeding symptoms are very common. A relationship between low VWF and bleeding can be difficult to establish. Genome wide linkage analysis has, to date, not identified an influence other than the ABO blood group, with small effects from the “Secretor” locus and the VWF gene itself. Two-thirds of the variance in VWF level cannot be attributed to genes. A reduced VWF level in heterozygotes for type 3 VWD confers only a modestly increased risk of bleeding. About 9% of women with menorrhagia are reported to have <50% VWF, which means that 90% of women with menorrhagia have normal levels of VWF. “What distinguishes the subjects with low VWF who are in the hemostasis clinic from the 99.9% of them who are not? A considerable body of data ... suggests that low VWF, from about 15% to 50%, rarely is associated with medically important bleeding.” However, the ascertainment of patients because they actually have bleeding, rather than by the discovery of a low VWF level, could select a subgroup with additional independent risk factors, some of which might interact with a low VWF level to increase the risk of bleeding.


On behalf of the ISTH committee on VWD, “type 1 VWD” was defined as an inherited bleeding disorder due to quantitative deficiency of VWF. For diagnosis, a patient must have significant mucocutaneous bleeding, laboratory tests compatible with the diagnosis, AND either a positive family history or an appropriate VWF mutation. The term “possible VWD type 1” may be used to describe persons with laboratory tests compatible with type 1 VWD and either significant mucocutaneous bleeding OR a positive family history. Alternative diagnoses should be sought for persons with “possible VWD”. This recommendation for mutation analysis is outdated; later studies showed that mutations cannot always be found in familial type 1 VWD.


In four families with highly-penetrant, moderately severe VWD, due to two different mutations, the half-life of VWF in affected persons was greatly reduced whereas the half-life of the propeptide was normal. Measurement of the ratio of propeptide to mature antigen distinguishes this pathophysiologic mechanism of type 1 VWD. This variant was subsequently called type 1C, “C” for clearance.


In a sample of 19 type 1 patients, the mean VWF:Ag half-life was 3.6 hours, with a wide range of 0.3 - 12.7 hours. The mean half-life of the propeptide was 3 hours with a range of 1.3-6.2 hours. DDAVP response correlated with the VWF propeptide/VWF:Ag ratio. Seven patients had an increased ratio (5.2 to 11.5) and a decreased VWF half-life (0.9-2.7 hours), also called increased clearance. Three other patients had a decreased VWF:Ag half-life (0.3-2.9 hours) not predicted by the propeptide/VWF:Ag ratio. However, treatment of an acute hemorrhage with DDAVP may be satisfactory regardless of the VWF half-life.

The same missense mutation was found in ten of the 70 families with type 1 VWD studied in Canada and in two additional UK families. Haplotypes in the 12 families suggest a remote common ancestry. Inheritance is dominant, incompletely penetrant.


The type 1 VWD phenotype was linked to the VWF gene locus in only 41% of families. Other genetic loci or non-genetic factors must play a role in pathogenesis.


The Canadian study of type 1 VWD recruited 150 index cases consistent with the diagnosis, then eliminated those with abnormal multimers, a low ratio of VWF:RCo/VWF:Ag, or a mix of type 3 and type 1 phenotypes, leaving a cohort of 123 affected index cases with 229 affected individuals in their families. Index cases had mean levels of VWF:Ag level of 36% and VWF:RCo of 34%. Blood group O was found in 50% of index cases with VWF:Ag levels of 30% or less and in 66% of those with higher levels. Fifty different mutations were found, 12 of which occurred in multiple index cases, accounting for 51% of index cases. The most common change was Y1584C, found in 15% of families, many from Quebec. Seven mutations were demonstrated to be completely penetrant, present in affected family members and absent in unaffected ones. No mutation could be found for three index cases with VWF:Ag levels below 20%. Missense mutations (n=31) were found throughout the gene; two were in the propeptide and two in codons 854 and 924 previously associated only with type 2N VWD. Some missense mutations may, in fact, be neutral polymorphisms. Eight mutations were found in the promoter. More than one change was found in 21 index cases.

Goodeve A and many others. Phenotype and genotype of a cohort of families historically diagnosed with type 1 von Willebrand disease in the European study, Molecular and Clinical Markers for the Diagnosis and Management of Type 1 von Willebrand Disease. Blood 2007, 109:112-121.

In the large study in Europe, mutations were defined in 70% of 150 index patients previously identified as having type 1 VWD. Among the 38% of index cases with abnormal multimer structure found with a sensitive test (NOT the absence of HMW multimers), mutations were found in 95%. Among those with normal multimers, only 55% had gene mutations. A third of the index cases might actually have type 2 VWD.


Segregation analysis in 143 families with previously-diagnosed type 1 VWD suggested linkage in 70%. After exclusion of families with abnormal multimer patterns (as defined in the previous paper) the proportion of the remainder (with normal multimers) in whom linkage could be demonstrated was only 46%. (That is, in 54% of families with normal multimers, VWD was not related to a specific VWF gene. Their somewhat low levels of VWF plus bleeding symptoms may have been caused by other influences.)

Among 150 index cases of type 1 VWD in the European study, 57 had abnormal multimers, mostly subtle, and mutations were identified in 54 of these cases. Among those with abnormal multimers, 39 had some loss of high molecular weight multimers and patterns indicative of a multimerization defect, that is, a subtype of 2A, and 18 others had all multimers and sometimes extra-large multimers compatible with type 2M. Completely normal multimers were seen in 93 cases, and mutations were found in 51 of these patients. A VWF:RCO/ VWF:Ag ratio less than 0.7 was found in 51% of cases with abnormal multimers and only 12% of cases with normal multimers. Thus, multimer analysis, although it is a complex technology, is important to the correct classification of VWD. The proportion of supposed type 1 cases diminishes further if expert multimer analysis is available: Dr. Budde’s lab is the most expert in Europe.


When a gene is found to have a change from the usual sequence, is that a normal variation or a mutation? Experience with the VWF gene is still in its infancy. Transfection experiments were performed with mutant VWF cDNA from 14 patients with missense mutations from the above study and from controls. Seven mutations led to marked intracellular retention and impaired secretion of VWF and major loss of high molecular weight VWF, thus these clearly were pathogenic mutations. Four other mutations were probably causative, with mild reduction in secreted VWF or with faster-running multimeric bands. For three mutations, transfected cells produced VWF indistinguishable from normal; one of these mutations tracked with VWD within the family, and is likely to be causative.


In the European study of type 1 VWD, the ratio VWFpp/VWF:Ag and the ratio FVIII coagulant activity /VWF:Ag were increased among patients with type 1 VWD compared with unaffected family members and normal controls. The ratio VWFpp/VWF:Ag was higher in individuals heterozygous for missense mutations (reflecting faster clearance of abnormal VWF) than in those heterozygous for null mutations (reflecting reduced synthesis). In contrast, the FVIII/VWF:Ag ratio was highest among heterozygotes for VWF null alleles, also reflecting reduced synthesis. Missense mutations also can cause combined defects of reduced synthesis and increased clearance.


These Belgians, not part of the European study, took a fresh look at type 1 VWD. “Autosomal dominant type 1 VWD variants are in fact type 2 variants caused by a heterozygous missense mutation...that produces a mutant VWF protein that has a dominant effect…” that is, dominant negative, impeding the synthesis or processing etc of normal VWF
from the other gene. “There will be cases of mild and moderate recessive type 1 VWD due to double heterozygosity of two missense mutations, or with the combination of one missense mutation with a non-sense or blood group O. Mild deficiency of VWF in the range of 0.20 to 0.60 U/mL with normal ratios...” of VWF:Ag, VWF:RCo and FVIII, with normal multimers and normal response to DDAVP “...are very likely cases of so-called pseudo-VWF deficiency...”

Peake I, Goodeve A. Type 1 von Willebrand disease. J Thromb Haemost 2007, 5 (suppl 1):7-11. (Highly recommended; three multicenter studies are reviewed.)

In three large studies, the lower the level of VWF:Ag, the more likely were multimers to be abnormal by a very sensitive test and the more likely was a mutation to be found; these patients also were likely to have a slightly reduced VWF:RCo/VWF:Ag ratio. In those subjects with <10% VWF:Ag, about 85% have multimer abnormalities and 95% have a detectable mutation. They might be reclassified as type 2 cases. At the other end of the spectrum, in index cases with VWF:Ag levels of 50% or more, multimers were normal in 95% of cases and putative mutations were found in only 48%. In linkage studies, in type 1 VWD with normal multimers, the disease phenotype co-segregated with the VWF gene in only 50% of cases. Blood group O was present in 63% of index cases where complete co-segregation was seen but in 89% of cases in the incomplete co-segregation category. Note that it is possible to have mutation-determined VWD with borderline factor levels, and, in families with obvious VWD and co-segregation, mutations may not be found.


The diagnosis of type 1 VWD is hampered by the lack of a good biological assay to assess the risk of bleeding associated with VWF deficiency. Decreased levels of VWF:RCo are associated with increased bleeding risk but the relationship is not strong. The test is not done under physiological conditions (that is, not under shear stress). In many individuals, it is difficult to confirm or exclude the diagnosis of type 1 VWD. Bleeding symptoms and low VWF levels overlap with normality.

In the three large studies of type 1 VWD (Canada, EU and UK) candidate mutations were identified in about 60% of supposed type 1 index cases. Up to 18% of patients had more than one mutation. Mutations associated with type 1 VWD were missense in 75% of instances and null in 25%, whereas in type 3 VWD, about 80% of mutations are null. The likelihood of finding a VWF gene mutation increases with decreasing levels of VWF.

Certain mutations are found recurrently in all the recent studies. One is Y1584C, found in 15% of Canadian and 8% of EU index cases, associated with blood group O in 95% of UK cases, and found in about 1% of the healthy population. In the UK, the combination of Y1584C and blood group A was associated with a reduction of 21% in the VWF:Ag level expected in non-mutant individuals, but that mutation combined with blood group O was associated with a reduction of 39% in the expected VWF:Ag level, a synergistic effect. Among people who had inherited both Y1584C and blood group O, 35% had a VWF:Ag level below 50%. In these patients, the VWF propeptide/VWF:Ag ratio was increased, compatible with increased clearance. Y1584C is associated with enhanced VWF susceptibility to proteolysis by ADAMTS13.

Routine mutation screening is not likely to improve the diagnosis of type 1 VWD because patients with mutations tend to have lower VWF levels and are relatively easy to diagnose. Even when VWF gene sequence
changes are found, their pathogenetic significance is often unclear.


These investigators in Chile compared the laboratory criteria for the diagnosis of VWD of four authorities on their lab studies of 4425 patients, referred for bleeding problems, over a 5 year period. The criteria were: (1) NHLBI, using a cutoff point of 30% for VWF:Ag and VWF:RCo, below that a diagnosis of VWD is made, and between 30% and 50% one may say “possible” VWD; (2) a modification of the NHLBI criteria allowing a diagnosis of type 1 if levels of both VWF:Ag and VWF:RCo were equal to or below 2.5th percentile in a person with normal multimers; (3) the European Group on VWF, EUVWD, using a level of VWF:RCo or VWF:CB less than 40%; and (4) the Zimmerman Program for the Molecular and Clinical Biology of VWD (Blood Bank of Wisconsin) using a level of either VWF:Ag or VWF:RCo of less than 40%. These criteria had a high correlation and excellent agreement but the number of patients diagnosed with VWD depended on criteria, with an increase from 2.8% to 8.3% in diagnostic rate mostly explained by increasing the cut-off values of VWF measurements of from < 30 to <40%.


Commenting on the above article, Dr. Rodeghiero favors the “Bayesian” approach, assuming a prevalence of VWD of 1 in 1000, and associating each component of the diagnosis (bleeding history, laboratory values and inheritance) with a post-test probability of being or not being affected by VWD. Each component could be set such as to obtain a higher or lower final probability of making a true diagnosis, while not missing relevant cases.


Commenting on Dr. Rodeghiero’s comment, the authors returned to their data and selected those 280 patients with unequivocal pathologic bleeding (bleeding score of 4 or more and a positive family history in 81.3%). In these patients, a diagnosis of VWD type 1 was made in 11.4% by NHLBI criteria, 22.5% by EUVWD criteria and 19.6% by the Zimmerman program criteria. A diagnosis of “possible VWD” was allowed in a further 21.1% by NHLBI criteria. These authors find that a comprehensive personal interview is as useful as the bleeding score in evaluating patients, but that a bleeding score may be a useful research tool. Mild type 1 VWD is often hard to “prove”. Use of qualifiers such as “probable” and “possible” is common among clinicians, who may prefer clinical judgment to the mathematical approach. On the other hand, when a bureaucracy demands a substantiated diagnosis, a clinician might need to choose some “official” criteria.

Type 1 VWD and platelet function defects

The adhesion of flowing platelets to collagen depends not only on GPIb-VWF interaction but also on collagen interaction with the platelet integrin alpha2 beta1. The gene for that integrin has at least three alleles, of which two are associated with low collagen-receptor den-
sity and one with high receptor density. The frequency of these alleles was similar in the normal population and in types 2A, 2B, 2M and 3 VWD. The frequency of the alleles associated with low receptor density was significantly higher in type 1 VWD. In patients with type 1 VWD and borderline levels of VWF:RCo, collagen-receptor density correlated with closure time in a PFA-100® analyser. Low density of the platelet collagen-receptor integrin may be associated with a mild tendency to bleed excessively. Differences in these platelet-gene alleles may account for some of the variability in clinical bleeding in patients with similar plasma clotting factor levels.

Dr. Weiss reviewed the records of 87 VWD patients with VWF:Ag or VWF:RCo levels between 15 and 50%, whose collagen-induced platelet aggregation had been studied, and scored their bleeding symptoms. Those with abnormal collagen-induced platelet aggregation tended to have had more bleeding. He wonders whether the common 807C allele within the α2 gene, associated with a low density of the α2β1 collagen receptor in platelets (required for normal collagen aggregation) may be inherited with determinants for a somewhat-low VWF level and result in a bleeding phenotype.


Bleeding severity in 14 unrelated VWD patients was correlated with platelet geno-

type. Significantly increased or decreased bleeding scores were demonstrated with certain haplotypes of three platelet glycoprotein genes that have single nucleotide polymorphisms, however, plasma levels of VWF:RCo or VWF:Ag had a stronger influence on bleeding scores.

Type 2 VWD


The A1 domain contains binding sites of platelet GP1b and minor sites for collagen. The major binding site for fibrillar collagen is in the A3 domain. A binding site for the GP IIb/IIIa complex is in the C1 domain. The FVIII binding site is at the D’ to D3 domain. Correspondence of phenotype (type of VWD) to particular domains is partly clear.


“Type 2A VWD appears to result from a complex intersection of mechanisms that include: (1) intracellular retention or degradation of VWF, (2) defective multimerization, (3) loss of regulated storage, and (4) increased proteolysis by ADAMTS13”.


Twenty patients with 2B VWD from
nine unrelated families were studied. In most, spontaneous thrombocytopenia had been recorded on at least one occasion. Three different point mutations were identified. One asymptomatic man, who was the son and the father of symptomatic persons, had the same gene mutation as his relatives but had completely normal laboratory tests. Laboratory tests in 11 symptomatic persons from nine families showed prolonged Ivy BTs and enhanced RIPA; other results were:

<table>
<thead>
<tr>
<th>Test</th>
<th>n</th>
<th>Range</th>
<th>Median, extrapolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVIII</td>
<td>12</td>
<td>22-64%</td>
<td>42%</td>
</tr>
<tr>
<td>VWF:Ag</td>
<td>12</td>
<td>32-68%</td>
<td>37%</td>
</tr>
<tr>
<td>VWF:RCo</td>
<td>11</td>
<td>5-33%</td>
<td>10%</td>
</tr>
<tr>
<td>VWF:CB</td>
<td>11</td>
<td>4-42%</td>
<td>12%</td>
</tr>
</tbody>
</table>


In all affected persons in this survey, RIPA was enhanced and HMW VWF multimers were reduced. Plasma levels are given below; note that the ratio of VWF:Ag/VWF:RCo is about 0.8, above the usual diagnostic guide for type 2 VWD. Without multimers or RIPA or low platelet counts, one might suspect type 1 VWD.

<table>
<thead>
<tr>
<th>Test</th>
<th>N</th>
<th>Range</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVIII</td>
<td>10</td>
<td>9-31%</td>
<td>14%</td>
</tr>
<tr>
<td>VWF:Ag</td>
<td>10</td>
<td>6-16%</td>
<td>9.5%</td>
</tr>
<tr>
<td>VWF:RCo</td>
<td>10</td>
<td>6-13%</td>
<td>8%</td>
</tr>
</tbody>
</table>


The “ristocetin cofactor is measured with a constant and non-limiting concentration of ristocetin that produces maximal VWF binding to platelets, an experimental condition that obliterates the enhanced function of type IIB VWF.” The paradox in type 2B VWD is that the patients had “a decreased concentration of ristocetin cofactor, and yet a lower threshold for the induction of RIPA…”


Variable phenotypes were found in 67 type 2B patients from 38 unrelated families, representing 11 different mutations. The diagnosis was defined as enhanced RIPA. Multimer patterns were heterogeneous. The risk of bleeding was higher in patients with thrombocytopenia, present in 20 patients at baseline and in 58 after stress (infections, surgery). Platelet counts and multimer patterns were always normal in 16 patients from five unrelated families. The authors conclude that that RIPA is still needed for diagnosis.


Ten patients with VWD from two apparently-unrelated families in Vicenza province, Italy, had mild bleeding problems with autosomal dominant inheritance. VWF multimers of larger-than-normal size were seen in plasma (but not in platelets). Affected persons had normal platelet counts, normal to borderline BTs and reduced RIPA. Other results were as shown in the table that follows:

The utility of two functional assays, VWF:RCo and VWF:CB, for differential diagnosis of VWD types were compared in 25 patients with type 2M VWD, six with type 2A and one with type 2B. VWF:CB was deficient in types associated with the loss of HMW multimers and was less deficient in type 2M in which there was defective platelet binding but normal-sized multimers. The authors concluded that VWF:CB should be used as an additional test, not a replacement for VWF:RCo. (Where available, VWF:CB may be useful to distinguish type 2M from other type 2 variants.) Mean test results were as follows:

<table>
<thead>
<tr>
<th>Test</th>
<th>N</th>
<th>Range</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVIII</td>
<td>7</td>
<td>15.1-45.0</td>
<td>20</td>
</tr>
<tr>
<td>VWF:Ag</td>
<td>7</td>
<td>6.25-32.0</td>
<td>14</td>
</tr>
<tr>
<td>VWF:RCo</td>
<td>7</td>
<td>7.1-29.6</td>
<td>11.1</td>
</tr>
<tr>
<td>VWF:CB</td>
<td>5</td>
<td>7.15-29.0</td>
<td>13.5</td>
</tr>
</tbody>
</table>

Bleeding symptoms were typical of VWD including nosebleeds, menorrhagia, bruising and post-surgical bleeding. Three patients had hemarthroses, suggestive of hemophilia A. BTs were within normal limits. Multimer distribution was normal or close to normal. Binding of VWF to FVIII was below the limit of detection. Heterozygotes were asymptomatic but had intermediate levels of FVIII-VWF binding. After infusions of concentrates of FVIII without VWF, levels of FVIII rose only briefly. After infusions of VWF concentrate or of FVIII-VWF concentrate, the rise in FVIII levels was prolonged. Factor levels were as follows:

<table>
<thead>
<tr>
<th>Test</th>
<th>N</th>
<th>Range</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVIII (activity)</td>
<td>8</td>
<td>5-22 %</td>
<td>About 7.5 %</td>
</tr>
<tr>
<td>FVIII:Ag</td>
<td>6</td>
<td>5.5-27.5 %</td>
<td>About 7.5 %</td>
</tr>
<tr>
<td>VWF:Ag</td>
<td>8</td>
<td>55-150 %</td>
<td>100 %</td>
</tr>
<tr>
<td>VWF:RCo</td>
<td>8</td>
<td>65-100 %</td>
<td>About 100 %</td>
</tr>
</tbody>
</table>


Data from various reports on eight patients with Type 2N VWD is summarized.

<table>
<thead>
<tr>
<th>Type</th>
<th>N</th>
<th>VWF:Ag,%</th>
<th>VWF:RCo,%</th>
<th>VWF:CB,%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>22</td>
<td>97</td>
<td>92</td>
<td>98.5</td>
</tr>
<tr>
<td>2A</td>
<td>6</td>
<td>22.5</td>
<td>&lt; 5</td>
<td>5</td>
</tr>
<tr>
<td>2B</td>
<td>1</td>
<td>32</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td>2M</td>
<td>25</td>
<td>27</td>
<td>5</td>
<td>35</td>
</tr>
</tbody>
</table>


A test for VWF binding to FVIII was performed on 177 unrelated patients diagnosed as having hemophilia A and 199 unrelated patients diagnosed as having type 1 VWD. Type 2N VWD was detected in five patients previously thought to have hemophilia A and three previously thought to have type 1 VWD. One compound heterozygote had a severe phenotype with FVIII levels of 1-2%. Patients who appear to have hemophilia A but do not have a sex-linked family history should be tested for VWF:FVIIIIB; nowadays, mutation testing for a hemophilia mutation might also be done.
Ribba AS, Loisel I, Lavergne JM et alia. Ser968Thr mutation within the A3 domain of von Willebrand factor (VWF) in two related patients leads to a defective binding of VWF to collagen. Thromb Haemost 2001; 86:848-854.

Two women in a family, heterozygous for the named mutation, had a bleeding disorder characterized by borderline BT and moderately decreased levels of VWF and FVIII. Multimer structure was normal. Binding to platelet GPIb was normal but binding to collagen was defective. (A singular defect in binding to collagen is unusual.)


Three different mutations were found in three families with defective binding of VWF to collagen. In transfection experiments, VWF produced by two of those mutations were associated with a pronounced binding defect to both type I and type III collagen but a third mutation was associated with defective binding to type I collagen only. A problem in assays for VWF:CB is the choice of collagen.

Type 3 VWD


Four families with VWD living in the Åland islands, including members of the original family described by von Willebrand, were investigated. One stop mutation was found in all four families. The only living patient with severe VWD was homozygous for that mutation. That mutation is common among patients with type 3 VWD in Sweden. Linkage analysis suggested that many Swedish patients were related to the Åland island patients.


The VWF gene was analyzed in 32 patients with severe VWD from 28 German families. A variety of mutations were found. There was little evidence of common ancestry. Complete deletions of the gene and nonsense mutations in the pro-sequence correlated with asymptomatic heterozygotes, whereas frameshift and nonsense mutations in the mature subunit correlated with a type 1 VWD phenotype in heterozygotes.


Among 40 patients with type 3 VWD from Italy, Iran and India, 50 gene defects were identified, of which 45 were novel. Most were null alleles. Mutations were scattered throughout the gene. No founder effect was seen in these countries.


A type 1 VWD phenotype was found in most of 55 subjects, heterozygous for a null allele, from 13 families with type 3 VWD with known mutations. With two of the mutations found, levels of VWF tended to be close to the lower limit of normal, whereas with a third mutation, levels of VWF were about half the lower limit of normal, that is, definitely abnormal; most of these persons had mild bleeding
symptoms. Genotype alone does not determine phenotype because individuals with the same mutation may vary greatly in VWF level, e.g., with one mutation, VWF:Ag levels varied from 13% to 110%, with another, VWF:Ag levels varied from 12% to 94%, and with a third, VWF:Ag levels varied from 13 to 39%.

The father of a girl with type 3 VWD was investigated. He had an almost-normal phenotype but a mutation on each of his two VWF alleles, one of which went to the girl with VWD, the other allele to each of his other three children, only one of whom had a mild VWD phenotype. The authors were impressed with the “silence” of the father’s mutations, and, therefore, studied 55 subjects who carry one null allele of the VWF gene, from families with type 3 VWD. The three different mutations and associated factor levels were as follows:

<table>
<thead>
<tr>
<th>Mutation on one gene</th>
<th>Mutation on one gene</th>
<th>VWF:Ag mean, %</th>
<th>Median, %</th>
<th>Range, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2680delC</td>
<td>2680delC</td>
<td>37</td>
<td>46</td>
<td>13-110</td>
</tr>
<tr>
<td>Arg1659X</td>
<td>Arg1659X</td>
<td>11</td>
<td>47</td>
<td>12-94</td>
</tr>
<tr>
<td>Arg1853X</td>
<td>Arg1853X</td>
<td>7</td>
<td>22</td>
<td>13-39</td>
</tr>
</tbody>
</table>

18.9 (3-60) in type 1 carriers. Mean levels of FVIII were 88.2% in type 3 carriers and 44.4% in type 1 carriers. Most type 3 VWD is caused by homozygous or doubly heterozygous null-alleles, and heterozygote carriers appear unaffected. Sometimes a heterozygote bears a type 1 VWD mutation, usually missense.


Mutations were identified in 33 (97%) index cases, and in 62 of 68 alleles. Of index cases, 17 were homozygous and 12 were compound heterozygotes. Three patients were homozygous for missense mutations and one was a compound heterozygote for a null and a missense mutations. Four index cases had a mutation identified on only one allele, a null mutation. In one index case, no mutation could be identified. Patients with mutations in the propeptide (42%) had more severe bleeding (bleeding score 22) symptoms than those with mutations elsewhere (bleeding score 13). Among obligate carriers, 48% had been diagnosed with type 1 VWD.


Type 3 families, n = 35, with obligatory carriers, n = 70, and normal controls, n= 215, plus 42 obligatory carriers of type 1 VWD (defined as having another affected first degree relative and an affected offspring), were studied. At least one bleeding symptom was reported by 23% of controls, 40% of obligatory type 3 carriers and 81.8% of type 1 carriers. Mean levels and ranges of VWF:RCo were 56.3 % (12-160) in type 3 carriers and

Alloantibodies in type 3 VWD


The development of alloantibodies against VWF occurs in up to 10% of type 3 VWD patients. Typically, it arises in multi-transfused patients with a partial or complete VWF gene deletion, often with a positive family history of such antibodies. Laboratory methods for antibody identification and characterization are inadequate.
**Pseudo-VWD (Platelet-type VWD)**


Four persons from four generations of a family had a mild bleeding disorder and intermittent thrombocytopenia. Plasma FVIII levels were borderline in two patients; VWF:RCo was low in two and borderline in a third. Large multimers of VWF:Ag were absent from plasma but present in platelets. RIPA was enhanced at low concentrations of ristocetin as in type 2B VWD, however, the disorder in this family was in the platelets, which adsorbed large multimers of VWF:Ag at lower concentrations of ristocetin than did normal platelets. Their platelets also aggregated in the presence of normal VWF without ristocetin.


A point mutation was found in the platelet GPIb gene from persons with pseudo-VWD. Affected persons were heterozygotes.


In 14 patients from five families, with a diagnosis of VWD but with no mutation in the VWF gene, pseudo-VWD was confirmed by finding a mutation in the platelet glycoprotein Ibα gene.

**Acquired von Willebrand syndrome**

The first case of VWD associated with an autoimmune disorder, as far as I can determine, was VWD associated with systemic lupus erythematosis, reported in 1968. Subsequently there were several reports of VWD with hypothyroidism or disorders of the lymphatic system. Later, starting about 1992, acquired VWD also was reported secondary to destruction of HMW multimers at areas of vascular obstruction such as stenotic valves. Reviews sometimes include both of these very different origins. These two types of acquired VWD should be distinguished from type 3 VWD with alloantibodies.


The literature up to 1999 contained 266 published cases of acquired VWD. An international survey added 186 patients. Underlying disorders included lymphoproliferative (48%) and myeloproliferative (15%) disorders, neoplasia (5%), immunological disorders (1%), cardiovascular disorders (21%) and others. Well-identified cardiac lesions included valvular stenosis or prolapse and septal defects. BTs tended to be prolonged, levels of VWF:RCo and VWF:CB tended to be low and were the most abnormal tests seen; levels of FVIII and VWF:Ag could be low to normal. In five tested patients, levels of the propeptide were normal, that is, higher than the patients’ reduced levels of VWF:Ag. HMW multimers were reduced in 83% of the tested patients. Inhibitory antibodies were demonstrated with mixing studies (e.g. levels of VWF:RCo performed on mixtures of patient and normal plasma) in only 16% of patients. Bleeding was mostly
muco-cutaneous. DDAVP stopped bleeding in 38 of 119 treated patients. FVIII/VWF concentrates stopped bleeding in 42/115 patients. High dose intravenous gamma globulin was effective (raising plasma FVIII-VWF levels after a delay of two-three days) in 21/63 treated patients. A majority of responsive patients (13/21) had neutralizing antibodies. Plasmapheresis with or without extracorporeal adsorption of gamma globulins was effective in 6/32 patients. Corticosteroids were effective in 12/63 patients and chemotherapeutic immunosuppressive agents in 23/66 patients.


Skin or mucosal bleeding was noted in 21% of 50 consecutive patients with aortic stenosis. Closure time in the platelet function analyzer was prolonged in 92% of those with severe stenosis and in 50% of those with moderate stenosis. HMW multimers were decreased in 79% of patients. In 42 patients having surgical correction, the platelet function analyzer CT and the multimer distribution corrected rapidly post-operatively.


A more sensitive ELISA test for antibodies to VWF was sought. Platelets were coated with purified VWF. Test plasma was added and incubated. Goat anti-human-IgG and –IgM antibodies, labeled with peroxidase, were added and incubated. A color reaction was developed to determine the uptake of antibodies. The test was able to detect antibodies (more commonly IgG than IgM) in eight of ten patients with acquired VWD whereas neutralization assay results were positive in only two of six patients tested. This test applies only to patients with autoimmune-type acquired VWD, not to that secondary to valvular obstruction.


A patient with monoclonal B-cell lymphocytosis and acquired VWD was treated successfully with anti-CD20 monoclonal antibody therapy (Rituximab®) as four weekly doses followed by the same dose every three months.


Prospectively, 53 patients answered questionnaires and underwent laboratory tests before an echo-cardiographic assessment of mitral regurgitation. Loss of high molecular weight VWF multimers was found in 8% of the 13 patients with mild regurgitation, 64% of the 14 patients with moderate regurgitation and 85% of the 26 patients with severe regurgitation. Only nine patients reported clinically-significant bleeding. Note that only a few of the affected persons were symptomatic. In the 20 patients who underwent mitral valve repair or replacement, all measures of VWF function improved significantly.
VWD and vascular lesions


The capillaries at the base of the finger-nails were observed through a binocular dissecting microscope. Experimentally, selected vessels were injured by inserting a glass fiber through the nail. In normal subjects the usual response to puncture of a capillary was hemorrhage lasting a few seconds, then disappearance of the vessel, presumably because it had been emptied of visible red cells. Dr. Macfarlane studied five women whom he believed had the condition described by von Willebrand. “The capillaries were studied with regard to reaction to puncture. They were of distorted and often bizarre forms, and did not contract after injury in any instance.” He differentiates this from telangiectasia “in which there is a sharply localized abnormality of the capillaries.”

The pictures below are reproduced from the original paper, above: normal vessels; below: VWD vessels showing increased tortuosity.


In Germany, capillaries in the nail skin-fold were studied with intravital microscopy in 100 normal subjects, 100 persons with VWD (92 type 1, 8 type 2A) and in a variety of other conditions. The subjects with VWD had significantly increased tortuosity of the capillaries (their pictures look almost identical to Dr. MacFarlane’s pictures), dilation of venules and arterioles, and extravasates from capillaries. The authors commented that the phenomenon had been neglected in recent decades.


Four patients with type 2A and two with acquired VWD are described, all of whom had gastrointestinal bleeding. Angiodysplasia was demonstrated in 5 patients and presumed in one (who had acquired VWD). Five patients had surgical procedures such as resections of affected areas. Estrogen-progesterone treatment was effective in two patients able to tolerate the hormones. The authors thought that VWD and angiodysplasia may not have a causal linkage, instead, persons who happened to have both conditions could be more likely to have obvious gastrointestinal hemorrhages. The opinion that it was all a co-incidence was prevalent until recent studies showed that VWF is needed for angiogenesis.

This review summarizes “the evidence that VWF controls angiogenesis, and describes the possible mechanisms through which VWF regulates angiopoietin-2 and integrin αvβ3, leading to signaling through vascular endothelial growth factor receptor-2 (VWGFR2), one of the most potent activators of angiogenesis.”


The evidence that VWF plays a role in angiogenesis is reviewed. Angiodysplasia is found only in VWD patients lacking HMW multimers, including VWD acquired due to shear stress, e.g. valvular lesions. Such lesions usually are multiple, and thus not easily treated with local measures. Infusions of VWF/FVIII concentrates are used, as are, on occasion, bypassing agents or platelet concentrates. Long-term prophylaxis with VWF-FVIII concentrates, e.g. 60 U/kg thrice weekly, has been useful.


In a retrospective study of 48 patients with congenital VWD and bleeding due to angiodyplasia, the condition was confirmed by endoscopy or surgery in 38%. All VWD types except 2N were represented. Prophylactic VWF/FVIII concentrate was the most effective treatment modality.

VWD and atherosclerosis


The aortas of 11 pigs with homozogous VWD were compared to those of 11 normal pigs of the same age, 1-3 years. Six normal pigs had multiple arteriosclerotic plaques and one pig had only one plaque. Of their total of 35 plaques, 24 were less than 2 mm in size and 11 were larger. Four VWD pigs had one lesion each, but only one of these lesions was more than 2 mm in size. In another experiment, 11 normal and 7 VWD pigs, aged 3 months, were put on a high-cholesterol diet for six months. All normal pigs developed extensive atherosclerotic aortic plaques whereas among the VWD pigs, four had no plaques and the others had fewer than seen in the control pigs.


Ultrasonography was used to measure intimal thickening and atherosclerotic plaques in the carotid and femoral arteries of 47 patients with type 3 (severe) VWD and 84 healthy controls. No difference between the groups was found.


In the Netherlands, 635 patients with VWD, VWF levels < 30%, aged 16-85, were compared to the general Dutch population adjusted age and sex. The prevalence of all arterial thrombotic events (including acute myocardial infarction, ischemic stroke and coronary heart disease) was 63% lower in VWD than in the general population. Perhaps VWD patients do not have a reduction of atherosclerosis but are protected by their state of hypocoagulability and reduced
thrombus formation. Their sample size was not large enough to permit analyses according to VWD severity or ABO blood groups.

**Symptoms**

Kouides PA. Females with von Willebrand disease: 72 years as the silent majority. Haemophilia 1998; 4:665-676. (Review, 110 refs)


A group of 81 women in the menstruating years who had type 1 VWD was compared to 150 control women of the same age group. A higher proportion of the women with VWD reported menorrhagia, anemia and hemorrhaging at delivery necessitating red blood cell transfusion. Hormonal treatment for menorrhagia was about 50% effective overall; high-dose oral contraceptive pills were only slightly more effective than standard dose pills. Menses had a much greater negative effect on quality of life (e.g. ability to go to work or school, participate in family activities, sleep) in women with VWD than in unaffected women.


In a retrospective review of 47 males (>90% under age 21) diagnosed with type 1 VWD in Pennsylvania, the most common bleeding symptoms were as follows: epistaxis in 52.6%; easy bruising 50%; postoperative bleeding 47.4%; hematomas 28.9%; dental or oral bleeding 28.9%; bleeding after minor outpatient surgical procedures in the mouth, nose or ears 21%; bleeding after trauma 15.8%; hemorrhhoses 7.9%; gastrointestinal bleeding 7.9% and hematuria 5.3%.


Among 75 adult women, average age 40 years, with type 1 VWD receiving care at hemophilia centers in the USA, the most common bleeding symptoms were as follows: menorrhagia in 84%, bleeding after tooth extraction 51%, bruising 48%, nosebleeds 44%, bleeding after injury 33%, post-partum bleeding 32%. The onset of bruising and nosebleeds was early, on the average, at age 7 years. The most common modality of therapy was DDAVP. Only 7% used blood products.

**Bleeding Assessment Tools (BATs), i.e. Bleeding Scores**


Bleeding symptoms were recorded retrospectively for patients and family members (n= 712) and controls (n=195) in the European study of type 1 VWD, using a questionnaire. Types and severities of various symptoms were scored, using numbers of 0-4 for no bleeding or increased bleeding and minus one for lack of excess bleeding in surgical procedures and deliveries, adding up to a summary bleeding score. Mean scores were 9 (range –1 to 23) in index cases with type 1 VWD, 4 (range -2 to 27) in affected family members, zero (range -2 to 14) in unaffected family members, and –1 (range -3 to 4) in normal controls. Higher scores were correlated (roughly) with lower levels of VWF and FVIII.

**Standardized bleeding assessment tools (BAT)** are “an effort to: (i) improve diagnostic accuracy and thus avoid unwarranted laboratory testing; (ii) predict the risk of bleeding in an individual patient; (iii) describe symptom severity; and (iv) inform treatment.” They can be used to screen individuals being investigated for an inherited bleeding disorder and also “to act as a standardized way of describing disease characteristics and of assessing disease severity.” The first such score was described in 2005, and there have been at least ten subsequent modified scores, including pediatric scores and menorrhagia scores.


Bleeding scores show promise as a research tool, but their applicability in clinical practice remains uncertain. “These instruments were designed to be administered by trained clinicians…” The greatest clinical utility of bleeding scores lies in their high negative predictive value, for those patients old enough to have faced hemostatic challenges.


Data from the original Vicenza bleeding questionnaire and subsequent bleeding scores, on 1040 normal adults and 328 children, were merged, using a statistical tool, to establish a current normal range, namely, 0-3 for adult males, 0-5 for adult females and 0-2 in children of either sex. Higher bleeding scores may be considered abnormal.

**Measurement of Menstrual Blood Loss**


Menstrual blood loss was quantitated by the alkaline hematin method in sanitary tampons and napkins carefully collected from women ages 15-50 in Sweden. The mean blood loss in 387 healthy women who believed their menstruation to be normal was 38.5 ml. In a group of 37 other women who believed their menstruation to be abnormal the mean blood loss was 100.7 ml. Women were asked whether they considered their blood loss to be scanty, moderate or heavy. In the group with the highest blood loss, exceeding 80 ml, 37% of women considered their blood loss to be moderate and 4% scanty. In the group with the lightest blood loss, less than 20 ml, 14% of subjects considered it heavy. (Objective criteria obviously were needed.)


In England, menstrual blood loss was studied in thirty normal women who filled out a pictorial blood loss assessment chart (PBAC) tallying the number of sanitary napkins and tampons used and the degree that each was saturated with blood, and who returned all used sanitary material to the study center so that blood loss could be measured objectively by the alkaline hematin method. There was good correlation (r=0.847) between blood loss and PBAC score, validating the accuracy of the PBAC.

Janssen CAH, Scholten PC, Heintz APM. A simple visual assessment technique to discriminate...

In The Netherlands, the PBAC score was compared to blood loss measured on all sanitary wear with the alkaline hematin method in 288 subjects. The two measurements correlated reasonably well (r=0.56) but not as well as in Higham's study. Two consecutive menstrual cycles were tested in 201 women; the degree of bleeding in the second cycle was similar to the first. The authors concluded that the PBAC had a good level of reliability and that scoring one cycle sufficed.


Women (n=217) aged 18-50, with a menstrual pictorial blood assessment chart (PBAC) score of 100 or more, answered a short bleeding screening tool and underwent coagulation testing including platelet function, PFA, VWF and other clotting factor assays. Abnormal coagulation tests were found in 71%; five percent had low VWF. The screening tool with or without the PBAC had a high sensitivity but low specificity for the diagnosis of a bleeding disorder.

Menorrhagia in VWD


A PBAC score of > 100, confirming menorrhagia, was found in 74% of women with VWD. The group included 59 women with type 1 VWD, four with type 2, and three with type 3. Higher PBAC scores were found in women who had < 30% VWF:RCo compared to women with higher VWF:RCo levels, not statistically significant.


Among adult women with type 1 VWD in Pennsylvania, 93.1% had menorrhagia. In over half the women, menorrhagia had been the first bleeding symptom in their lives, and had started at menarche.


Menorrhagia is reported in 74% of women with VWD and VWD is found in 13% of women with menorrhagia.


In a retrospective review of eight years' experience in a Children's Hospital (with an excellent coagulation laboratory), 71 girls aged 10 to 19 years presented with menorrhagia and only two proved to have type 1 VWD (and some had other disorders.)


Women referred from the Department of Gynecology to that of Haematology at the All India Institute of Medical Sciences (New Delhi) over a nine-year period of time were reviewed. Of 2200 women referred, 337 were found to have inherited bleeding disorders, most frequently platelet disorders (283 cases). VWD, in 40 women, was the most common plasma clotting factor disorder. (Restated, about two cases of VWD were found per 100 symptomatic women).

Excessive bleeding symptoms were reported in 74% of 102 women with VWD registered in hemophilia treatment centers versus 6% of 88 female controls. Women with VWD reported a highly statistically-significant greater frequency of post-partum bleeding, other gynecological conditions, arthritis and migraine headaches. Although menstrual problems interfered with life activities in the women with VWD, the prevalence of depression was not elevated.


Women with VWD are not only more susceptible to menorrhagia than control women, but also have a higher incidence of ovarian cysts, endometriosis, fibroids and polyps, miscarriage and postpartum hemorrhage including vulvar hemorrhage after vaginal delivery.

Pregnancy in VWD


The authors reviewed 24 pregnancies in 13 patients of whom seven had type 1 VWD and six type 2A or 2B. Some deliveries were managed with prophylactic infusion of cryoprecipitate or FVIII-VWF concentrate or, in one instance, DDAVP. They recorded hemorrhages at the time of delivery (primary) and those occurring days later (secondary, see table below). Hemorrhage was more likely in type 2 patients especially if no prophylaxis had been given, as follows:

<table>
<thead>
<tr>
<th>VWD type</th>
<th>Prophylaxis</th>
<th>Primary hemorrhage</th>
<th>Secondary hemorrhage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>YES</td>
<td>0/5</td>
<td>05</td>
</tr>
<tr>
<td>1</td>
<td>NO</td>
<td>0/8</td>
<td>3/8</td>
</tr>
<tr>
<td>2</td>
<td>YES</td>
<td>0/5</td>
<td>2/5</td>
</tr>
<tr>
<td>2</td>
<td>NO</td>
<td>3/6</td>
<td>1/6</td>
</tr>
</tbody>
</table>


Eighty-four pregnancies in 31 women with VWD (27 type 1, two type 2, two type 3) seen at one hospital over 17 years were reviewed. There were 18 spontaneous miscarriages and 12 elective abortions, with excessive bleeding requiring blood transfusion in three instances. Postpartum hemorrhage occurred in ten of 54 deliveries in women with VWD, necessitating blood transfusion in six instances (types of VWD not identified). Secondary hemorrhage was reported in 11 of 54 deliveries, with three instances of blood transfusion. No postpartum hemorrhage occurred in the 10 women with VWD who had been given prophylactic treatment at the time of delivery.

The authors reviewed 385 patients of both genders with type 3 VWD in Iran, 67% of whom were born to known consanguineous marriages. Menorrhagia was reported in 90/130 women of childbearing age (69%). The miscarriage rate was similar to that of the general population. At delivery, women usually were treated with plasma, cryoprecipitate or FVIII-VWF concentrates. Postpartum hemorrhages (requiring delay in hospital discharge or red cell transfusion) were reported in only 15/100 parous women. Abnormal bleeding usually occurred when this treatment was given for too short a period of time (for one day instead of 3-4 days). (It is remarkable that 31% of the women with type 3 VWD did not report menorrhagia and that post-partum hemorrhages were not more common; one wonders whether some women had a lesser bleeding tendency than is usually associated with type 3 VWD, or whether they were loathe to admit to medical problems.)


Levels of VWF:RCo and VWF:Ag peak within a few hours after delivery in normal women and in women with VWD, and thereafter fall rapidly, approaching baseline at one week and reaching baseline at three weeks. Women with VWD may be at risk for postpartum hemorrhage.


The authors reviewed 185 deliveries to women who had VWD or were hemophilia carriers, between 2002-2011, managed in the Netherlands according to current guidelines. Primary post-partum hemorrhage (500 ml blood loss or more within 24 hours) was observed in 62 deliveries (34%) 14 of which had > 1000 ml blood loss. Some of these hemorrhages occurred despite use of clotting factor concentrates during labor. Target factor levels around 100% had been used but the authors suggest that perhaps a target of 150-200%, similar to levels observed in normal women at term, might be appropriate.

Epidural anesthesia for delivery

I found no series, but three case reports. One woman (VWD type not described) got DDAVP 50 minutes before delivery and did well. Another with a probable type 2 did well with no blood products or DDAVP. A third with type 2A received FVIII-VWF concentrate before anesthesia and did well.

DIAGNOSTIC TESTS

Reviews on choice of tests


More than 90% of samples sent to the author’s referral center for VWD testing were shipped from other towns. A high proportion of shipped-in samples had decreased VWF:CB and loss of HMW multimers. Repeat, fresh samples rarely showed any abnormal-
ity. Samples obtained locally at his center had a low frequency of decreased VWF:CB. Holding a blood sample un-centrifuged at 4°C, or holding separated plasma at 2-4°C for some hours before freezing, resulted in loss of HMW multimers. Blood samples for coagulation tests should be processed quickly. This is a very pertinent observation. Ideally, the patient should go IN PERSON to an expert center with its own well-standardized laboratory.

Favaloro EJ. A duplex issue: (i) time to re-appraise the diagnosis and classification of von Willebrand disorder, and (ii) clarification of the roles of von Willebrand factor collagen binding and ristocetin cofactor activity assays. Haemophilia 2002; 8:828-833.

An excellent algorithm for the laboratory diagnosis of VWD was offered. Dr. Favaloro relied on the VWF:CB test, popular in Australia, as the first quantitative functional test for VWD. If abnormal, he then also measured VWF:RCo. If the two functional tests (VWF:CB and VWF:RCo) were notably lower than VWF:Ag, he presumed type 2 VWD and then recommended RIPA to discriminate 2A and 2M (low RIPA) from 2B and pseudo-VWD (hyper-RIPA). If RIPA was low, he advised multimer analysis, to distinguish true 2A with absent large multimers from 2M with no loss of large multimers. Multimer analysis was delegated to a late stage, perhaps reflecting the test’s poor availability. He advised that tests should be repeated when they are borderline or do not correlate with clinical observations or otherwise do not make sense.


The assay for VWF ‘activity’ based on a monoclonal-antibody (mAb) directed against a functional epitope on VWF (called “VWF:Act” at the time) using latex agglutination, has become popular. However, in this study, the VWF:RCo and VWF:CB assays had higher sensitivity to the loss of high-molecular weight VWF than did the “VWF:Act” assay. Some VWF:CB assays were more sensitive than others but all were more sensitive than “VWF:Act”.


Laboratory tests reflecting VWD were followed over one menstrual cycle in 15 normal young women. In three women, VWF:RCo or VWF:Ag sometimes fell below the normal range. Lab results in one woman were abnormal at the beginning of the menstrual cycle but within normal limits at the end. This paper has been quoted often, and for some years, a tested woman’s time in her cycle was recorded. Nowadays, a borderline lab value is unlikely to be repeated in order to catch it being just above or below some arbitrary limit of normal.


In persons with an appropriate personal and/or family history of bleeding, general tests should include prothrombin time, APTT, platelet count and PFA-100 or bleeding time. Levels of VWF:Ag, VWF:RCo and FVIII should be measured from the same sample of blood (because of possible variation in the individual from time to time). Depending on the ratios found, further tests might include RIPA, multimers and/or genetic analysis. In Europe, about half of labs still use the classic
VWF:RCo method and half use VWF activity tests, i.e. assays determining GPIb binding. These are not yet well-regarded by everyone but do show promise. One advantage is that they are not affected by the polymorphism that leads to abnormal VWF:RCo readings in some black subjects.


Bleeding times

Duke WW. The relation of blood platelets to hemorrhagic disease: Description of a method for determining the bleeding time and coagulation time and report of three cases of hemorrhagic disease relieved by transfusion. JAMA 1910; 55:1185-1192. Original description of Duke BT.


Ivy AC, Nelson D, Bucher G. The standardization of certain factors in the cutaneous "venostasis" bleeding time technique. J Lab Clin Med 1941; 26:1812-1822. The Ivy BT described above was modified.


Four patients with symptoms suggestive of VWD had normal BTs but two hours after ingesting 0.65 grams of aspirin the BTs were mildly prolonged in two and markedly prolonged in the other two. (Baseline levels of FVIII were 5 %, 9% and 50% respectively in three patients and not determined in the fourth.) In four patients with hemophilia A, the BT also was normal before ingesting aspirin but, in three of the four, was prolonged afterwards. Dr. Quick points out that his “aspirin-tolerance test” is helpful in unmasking some but not all questionable diagnoses of VWD. The aspirin tolerance test was popular for a while. Dr. Quick’s observations also led to the following study.


The authors used a template and scalpel blade to make a cut of one mm depth and 6 mm width on the forearm. Baseline Ivy BTs were determined on 60 normal adult males who then ingested (double-blind) capsules containing either one gram of aspirin or a placebo. After two hours the BT was repeated. The mean baseline BT was 5 minutes, range 2.5 to 10. The mean BT after placebo was 5.5 minutes and after ASA was 9.5 minutes, maximum of 16 minutes. The post-aspirin BT exceeded 10 minutes in 16 of 30 subjects. In some subjects who had ingested aspirin, larger-than-usual drops of blood were collected on the filter paper, thus, aspirin induced more profuse as well as more prolonged bleeding in those persons.


The baseline Ivy BT was within normal limits in nine of 11 patients with severe he-
mophilia A and all eight with severe hemophilia B and after aspirin ingestion was prolonged (beyond the normal post-aspirin range established in the above paper) in 12 of the 19 patients. Seven patients required clotting factor replacement to stop the bleeding. Patients with mild hemophilia A and B, and with moderate to severe factor XI deficiency, had normal baseline BTs and responded to aspirin like normal subjects. Baseline BTs were mildly prolonged (12 and 17.5 minutes respectively) in two of three patients with moderate VWD and were notably longer (20 and 40+ minutes respectively) after aspirin; in the third patient, the BT lengthened from 6 to 15.5 minutes. This study helped establish that aspirin should not be given in hemophilia.

**Platelet adhesiveness**

This now-obsolete test is mentioned in many reviews. It was very useful in its day. It fell out of favor because the apparatus was tedious to make in a clinical laboratory and difficult to standardize on a commercial basis.


A platelet count was made on anticoagulated venous blood obtained directly from a vein. Venous blood also was allowed to flow from a needle in the vein into polyvinyl tubing (internal diameter 0.113 inch) filled with one gram of glass beads, 0.0185 inch diameter, and thence into a test tube with anticoagulant for a platelet count. The latter count was compared with the direct venous platelet count to determine what percentage of platelets had adhered to the glass beads. The normal range was 26-60% in 45 normal subjects. In 11 patients with VWD, the range was 0 to 28% (all results except one were 16% or less).

Salzman’s test, the Ivy BT and FVIII levels were used to investigate 14 persons with VWD and 219 family members. Salzman’s test was abnormal in 95 persons and, in 44 of these, was the only abnormal test. (The ristocetin cofactor test had not yet been developed.) It was abnormal in all persons with a prolonged BT or reduced FVIII. It was normal in persons with hemophilia A and B; with congenital deficiencies of fibrinogen, factor V + VIII, factor VII; with coumadin use. It was abnormal in a few other situations including anemia and renal disease. Salzman’s test was hailed as more sensitive than the BT.

**Platelet function analyzers**


Dade International Inc. developed an instrument, called PFA-100®, for measuring platelet function in citrated fresh whole blood. The instrument aspirates a blood sample under constant vacuum through a capillary tube and past a membrane to an aperture. The membrane is coated with Type 1 equine collagen and also with either epinephrine or ADP. The shear stress is at a rate of about 5000-6000 second⁻¹. When a stable platelet plug forms, it closes the aperture. The time required for aperture closure is called the “closure time”, CT. The instrument was developed to mimic the BT as a more-standardized test of platelet-plug formation.

In France, PFA-100® results were abnormal in all VWD patients (except type 2N) using the collagen-ADP cartridges and all but two using the collagen-epinephrine cartridges, as shown in the table below. The CT also was prolonged in three patients with (platelet-type) pseudo-VWD, two with Glanzmann’s thrombasthenia, four with storage pool disease, and six with an aspirin-like defect.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>N</th>
<th>VWF:Ag, %, range</th>
<th>VWF:RCo, %, range</th>
<th>CT, ADP, sec, range</th>
<th>CT, epinephrine, sec, range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>96</td>
<td>56-207</td>
<td>58-209</td>
<td>66-126</td>
<td>77-186</td>
</tr>
<tr>
<td>Hemophilia A/B</td>
<td>14</td>
<td>60-200</td>
<td>54-186</td>
<td>60-119</td>
<td>77-138</td>
</tr>
<tr>
<td>VWD type 1</td>
<td>36</td>
<td>9-62</td>
<td>5-39</td>
<td>127- &gt;250</td>
<td>137- &gt;250</td>
</tr>
<tr>
<td>VWD type 2A</td>
<td>10</td>
<td>30-92</td>
<td>&lt;3-43</td>
<td>All &gt;250</td>
<td>All &gt;250</td>
</tr>
<tr>
<td>VWD type 2B</td>
<td>2</td>
<td>51-61</td>
<td>15-17</td>
<td>Both &gt;250</td>
<td>Both &gt;250</td>
</tr>
<tr>
<td>VWD type 2N</td>
<td>2</td>
<td>67-83</td>
<td>54-74</td>
<td>81-94</td>
<td>135-141</td>
</tr>
<tr>
<td>VWD type 3</td>
<td>4</td>
<td>All &lt; 1</td>
<td>All &lt; 3</td>
<td>All &gt;250</td>
<td>All &gt;250</td>
</tr>
<tr>
<td>Acquired VWD</td>
<td>5</td>
<td>14-49</td>
<td>3-40</td>
<td>140 - &gt;250</td>
<td>166 - &gt;250</td>
</tr>
</tbody>
</table>


In London, the CT with each of the two cartridges was prolonged in 50 of 53 patients with well-characterized VWD, a sensitivity of 94%, whereas BTs were prolonged in only 58%. The CT was more prolonged in the presence of qualitative VWF defects but it was not useful in distinguishing among VWD types. The CT did not vary according to ABO blood groups.

Ristocetin- induced platelet aggregation (RIPA)


The ability of ristocetin to aggregate platelets in platelet-rich plasma in vitro was described. (The test described here, the first using ristocetin, is RIPA). Aggregation was normal in a patient with afibrinogenemia, normal to reduced in four patients with thrombasthenia, and absent in two of three patients with VWD. They probably had fairly severe VWD.

Dowling SV, Muntz RH, D’Souza S, Ekert H. Ristocetin in the diagnosis of von Willebrand’s disease: A comparison of rate and percent aggregation with levels of the plasma factor(s) necessary for ristocetin aggregation. Thromb Diath Haemorrh 1975; 34:465-474. Aggregation was measured by following the change in optical density of the platelet suspension in a cuvette.
Ristocetin cofactor (VWF:RCo)


A quantitative assay was devised by measuring the aggregation of washed normal platelets by ristocetin in the presence of normal or test plasma at various dilutions. A standard reference curve was devised by plotting the degree of aggregation (measured by optical density) against the dilution of normal plasma.


Normal platelets fixed in formalin proved to be a stable reagent.


Using formalin-fixed platelets, the authors measured ristocetin cofactor by time to agglutination (rather than by optical density) plotted against the dilution of plasma. The assay could be performed using ordinary test-tubes and the end-point, agglutination, could be observed with the naked eye, thus, no machine to read optical density was needed. (This simple assay remains suitable for minimally-equipped laboratories around the world.)


Platelets fixed in formalin for use in the ristocetin cofactor assay were stable if frozen or lyophilized (making the assay for more convenient, and making commercial kits possible. Kits are expensive, however, and pickled platelets can be prepared in-house.)


An automated coagulation analyzer which stirs the contents of the cuvette and reads optical density was used to assay VWF:RCo with good reproducibility. (Automation of part of the process made the VWF:RCo test more widely accepted.)

VWF Activity assays (monoclonal Ab)


A monoclonal antibody that bound to a VWF epitope involved in its interaction with GPIbα was used in a solid phase ELISA test. It was found to be sensitive and reproducible as a substitute for the ristocetin cofactor test. (Some other laboratories disagreed.)

VWF binding to collagen (VWF:CB)


VWF:CB assay appeared to be more sensitive and reproducible than VWF:RCo for diagnosing type 2 VWD and became the preferred functional test in Australia. Mean factor levels, %, (simplified from their table 1) were as follows:

<table>
<thead>
<tr>
<th>Subjects</th>
<th>N</th>
<th>VWF:RCo</th>
<th>VWF:CB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>50</td>
<td>102.1</td>
<td>99.7</td>
</tr>
<tr>
<td>type 1</td>
<td>30</td>
<td>32.8</td>
<td>35.2</td>
</tr>
<tr>
<td>type 2A</td>
<td>10</td>
<td>33.0</td>
<td>7.4</td>
</tr>
<tr>
<td>type 2B</td>
<td>12</td>
<td>18.9</td>
<td>8.1</td>
</tr>
</tbody>
</table>


In this Italian laboratory, the VWF:CB test was more sensitive than the VWF:RCo in the diagnosis of VWF type 2, as follows:

<table>
<thead>
<tr>
<th>n</th>
<th>VWF:Ag</th>
<th>VWF:RCo</th>
<th>VWF:CB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>42</td>
<td>97.9</td>
<td>102.5</td>
</tr>
<tr>
<td>Type 1</td>
<td>37</td>
<td>47.3</td>
<td>31.1</td>
</tr>
<tr>
<td>Type 2</td>
<td>16</td>
<td>34.9</td>
<td>11.9</td>
</tr>
</tbody>
</table>


VWF binding to factor VIII (VWF:FVIIIb)


A monoclonal antibody to VWF was coated onto wells of microtiter plates. Various dilutions of normal or patient plasma were added. Wells were washed to remove any FVIII associated with the VWF from the test plasma. Purified FVIII was then added to the well. The amount of bound FVIII was estimated directly in the well either by a chromogenic assay or by incubation with a radio-labelled antibody to FVIII. The relationship between the amount of FVIII bound per unit of VWF originally immobilized on the well was determined for dilutions of normal plasma to make a reference curve. Two siblings were tested, who had minimally prolonged BTs of 9-10 min, FVIII of 15 and 20%, VWF:Ag of 39 and 53%, VWF:RCo 39 and 54%, respectively. Binding of purified FVIII to their VWF was markedly decreased.


A quantitative ELISA assay used anti-VWF-coated plates incubated with diluted normal or patient plasma, then washed and incubated with exogenous recombinant FVIII. The amount of bound rFVIII molecule was detected with a peroxidase-coupled anti-FVIII antibody. In 50 normal subjects the range of 60-130 U/dl was observed. VWF:FVIIIb was absent to markedly decreased in subjects with type 2N VWD. In 50 normal persons, two 2N homozygotes and two 2N heterozygotes, the following values, in %, were found, as shown in the table at the top of the next page:
Von Willebrand factor antigen (VWF:Ag)


VWF:Ag could be quantitated by electrophoresing plasma samples in wells cut into an agarose gel plate (method of Laurell, 1965). Antibodies to VWF:Ag had been mixed into the agarose. After electrophoresis, plates were stained and the height of precipitin “rockets” was measured. A reference curve was prepared by electrophoresis of dilutions of normal plasma. (The method used before ELISA methods.)


For an ELISA test for VWF:Ag, microtiter plates were coated with a goat F(ab’) 2 antibody to VWF. After the VWF from a plasma sample was bound to the plate, the amount bound was quantitated using a horse-radish peroxidase-labeled goat Fab’ antibody to VWF. The test was more sensitive than previous ELISA methods to traces of VWF.

Multimers


Previous tests for VWF:Ag multimer distribution used radio-iodine I-125 to label the anti-VWF antibody. These authors substituted a peroxidase-labelled goat anti-rabbit IgG to label multimers.


"Molecular sieving electrophoresis of vWF multimers, particularly in agarose gel or polyacrylamide-agarose gel combinations, in the presence of sodium dodecyl sulphate (SDS) and urea, is” a standard technique. “A clearly demonstrable repeating triplet banding is…the minimum degree of resolution necessary to correctly classify subtypes of VWF...particularly those with only minor abnormalities in triplet patterns. This was obtained in our system at relatively low agarose concentrations....” “Systems with relatively low resolution demonstrate a series of logarithmically spaced bands differing by constant increments in molecular weight. Higher resolution techniques have shown that each multimer is composed of at least three to five sub-bands. “

<table>
<thead>
<tr>
<th>Subjects</th>
<th>FVIII</th>
<th>VWF: Ag</th>
<th>VWF: RCo</th>
<th>VWF: FVIIIb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>60-160</td>
<td>60—160</td>
<td>60-130</td>
<td>0.6—1.3</td>
</tr>
<tr>
<td>Homozygotes</td>
<td>8.3</td>
<td>40 - 48.5</td>
<td>48.7 - 49.2</td>
<td>0.17 - 0.21</td>
</tr>
<tr>
<td>Heterozygotes</td>
<td>55 - 76</td>
<td>29.3 - 33.1</td>
<td>21.9 - 38</td>
<td>1.2—1.3</td>
</tr>
</tbody>
</table>
TREATMENT


**Anti-fibrinolytic agents**


EACA was given at 6 hour intervals for total daily doses of 12-40 grams for one day prior to and 3 – 5 days after 31 dental extractions on 19 occasions in 11 different patients with hemophilia. Tooth sockets were packed and the areas splinted for protection. No plasma products were required for hemostasis.


In England, 12 consecutive patients with hemophilia had extractions of one to 16 teeth under cover of fresh frozen plasma. The average patient used 5,900 ml of plasma and 2.6 units of whole blood. Subsequently, another 12 consecutive patients with hemophilia had extractions of one to 16 teeth under cover of EACA. One patient needed a transfusion of 450 ml plasma and two units of whole blood, the other 11 patients required no blood products. (This paper and the preceding one established the utility of EACA for dental extractions in hemophilia. The drug also proved useful in VWD.)


EACA versus placebo were given in a double-blind trial to 37 women with menorrhagia. The dose of EACA was 18 grams on the first day of menses, then 12, 9, 6 and 3 grams, respectively, on subsequent days. The mean blood loss per menstrual period on EACA was 51.9 ml and on placebo was 126.9 ml. (The subjects had menorrhagia but no bleeding disorder was mentioned. That is also true for the next few papers.)


Tranexamic acid, in a dose of one gram four times a day, significantly reduced objectively-measured menstrual blood loss in a double-blind study of 16 women with menorrhagia.


Tranexamic acid reduced measured blood loss by 58% in women with menorrhagia. It decreased plasminogen activator activity in endometrial tissue, as studied with biopsies.


Tranexamic acid (one gram every 6 hours) reduced measured blood loss by 54% in 76 women with menorrhagia.

Four patients with menorrhagia and VWD (two type 2A, one type 2B, one type 1) were managed with once-daily tranexamic acid, which proved to be as effective as divided doses.


A woman with VWD (probably type 2), whose profuse, incapacitating menorrhagia had persisted despite oral contraceptive agents, responded well to four grams of tranexamic acid taken once daily for three days beginning on the first day of menses.


Three women with VWD (one type 1, two type 2A) with severe menorrhagia had not responded adequately to a dose of one gram of tranexamic acid per day in but they were managed successfully with a dose of three grams per day for the first five days of the menstrual cycle.


All VWD patients received tranexamic acid for one day prior and seven days after oral surgery. If three or more teeth were extracted, fibrin glue was applied locally.

DDAVP was given (0.3 ug/kg subcutaneously, 60 minutes before the extraction) to patients with type 1 and 2A VWD known to be responsive, but not to patients with type 2B VWD. FVIII-VWF concentrate was given to patients with types 2B or 3 VWD.

Tranexamic acid alone, or with fibrin glue, was given on 30 occasions, after which only one patient had post-extraction oozing, which responded to further applications of fibrin glue. DDAVP (plus the above therapy) was given on 66 occasions to patients with type 1 or 2A VWD, none of whom had post-extraction bleeding. Factor VIII-VWF concentrate (plus the above therapy) was given on 21 occasions to patients with type 2 or 3 VWD; only one had post-extraction bleeding.


An excellent review with special attention to controlled trials. The mainstays of management in the childbearing years are oral contraceptive hormones, anti-fibrinolytic agents and intranasal DDAVP. There have been no controlled trials on the effect of oral contraceptive pills (estrogen-progesterone combinations) on menstrual flow but the general impression has been that they decrease flow. Intrauterine devices that release progesterone suppress endometrial growth and decreases menstrual flow. After childbearing is complete, endometrial ablation or hysterectomy can be considered.

DDAVP

Reviews


Observations


A dose of adrenalin increased plasma levels of factor VIII to a mean of 176% of baseline in 12 of 13 normal subjects. Adrenalin induced an increase in FVIII in four of five subjects with mild hemophilia A; in one such individual, the level rose from 12% to 120%.


DDAVP was given intravenously to five normal men, resulting in a rapid increase of plasminogen activator in the plasma.


DDAVP in an intravenous dose of 0.3 micrograms (ug)/kg in four patients with moderate to mild hemophilia resulted in a two- to three-fold rise in plasma FVIII level. Two treated patients had dental extractions without excess bleeding. A higher dose (0.4-0.5 ug/kg) caused a more marked response and was used to provoke a rise in plasma FVIII level in six patients with hemophilia A and two with VWD for surgical procedures.


After an intravenous DDAVP dose of 0.4 ug/kg, FVIII levels in 50 normal subjects increased from a mean of 135% to a mean of 425%, peaking at 30-60 minutes after infusion. Elevations of VWF:Ag peaked at the same time but did not reach such high levels. Plasminogen activator levels rose and peaked immediately after infusion. In a patient with apparently-severe VWD, the FVIII level rose from 6% to over 40%, the plasminogen activator level rose (but not as much as in a normal person), but VWF:Ag did not rise. In two other patients, who had probably-homozygous severe VWD, DDAVP did not provoke a rise in FVIII, VWF:Ag or plasminogen activator. The authors proposed that synthesis of plasminogen activator was affected by VWD.


DDAVP-induced increases in plasminogen activator activity were maximal at 30 minutes post-infusion in normal subjects, with a T ½ of 300 minutes. The rise in plasminogen activator also was seen in patients with mild hemophilia and mild to moderate VWD, but not in one patient with very severe VWD nor in another with fairly severe VWD.
DDAVP produces a maximal response at a dose of 0.3 ug/kg given intravenously, with an approximate five-fold increase in FVIII levels, and a three-fold increase in VWF:Ag levels in normal persons, persons with mild hemophilia A and persons with (type 1) VWD. Tachyphylaxis was described, that is, in some normal subjects and in some persons with mild hemophilia A given daily doses of DDAVP, the response on the second and third day was similar to that on the first day and in other such subjects the response was less than on the first day.

The effect of intravenous DDAVP (0.2 ug/kg, a little less than used in later years) was studied in three normal persons, 18 persons with VWD who had no structural abnormality of VWF:Ag, and four persons with mild to moderate hemophilia A. All had marked increases in FVIII, VWF:Ag and VWF:RCo after receiving DDAVP. In six patients with VWD who had prolonged BTs at baseline, all were normal between 15 and 90 minutes after DDAVP. The drug was used to provide hemostasis in eight subjects (one with hemophilia A, seven with VWD) for surgical procedures (3 dental extractions, one other oral surgery, 3 tonsillectomies, 1 nasal polypectomy) with excellent hemostasis. (All but one patient were also given an antifibrinolytic agent as was routine for surgery in the mouth area.) DDAVP successfully provided hemostasis in two patients with hemophilia with soft tissue and joint bleeding and three with VWD and menorrhagia.

A normal human umbilical vein perfusion model was used. Platelet adhesion and spreading at sites of minimal injury, observed with scanning electron microscopy, was greatly increased in veins pre-perfused with DDAVP compared to buffer control. DDAVP may have a direct, local effect on platelet adhesion, which may explain its beneficial effect in some bleeding conditions, e.g. uremia, not associated with low levels of plasma VWF. Perhaps DDAVP stimulates release of VWF from local endothelial cells and the VWF binds immediately to the exposed subendothelium, encouraging platelet binding.

Human umbilical artery, denuded of the endothelial layer, was perfused with whole blood or blood components obtained from subjects before and after intravenous DDAVP to study the adherence of platelets to the subendothelium. Platelet adherence in normal persons or persons with type 1 VWD who had been infused with DDAVP was much enhanced over baseline. Platelet adherence was unaffected or only slightly improved after DDAVP in persons with type 2A VWD. Platelet adherence decreased after DDAVP in persons with type 2B VWD. (The implied prediction that DDAVP might not be useful in type 2 VWD, was partially borne out.)
DDAVP, 0.3 ug/kg intravenously, was given to 21 patients with VWD. Levels of FVIII tended to increase more than levels of VWF:Ag or VWF:RCo. A prolonged BT was fully corrected in eight patients and partially corrected in two more. Individuals tended to have similar responses to DDAVP on each occasion of its use (if separated by a few days) and family members with VWD tended to have similar levels of response to DDAVP.


In three patients with type 2A VWD, infusion of DDAVP resulted in normal BT, FVIII, VWF:Ag, VWF:RCo and a normal multimer pattern if the blood samples were collected in protease inhibitors. Without such inhibitors, fewer of the intermediate and large multimers were seen. The authors conclude that the VWF in these patients is easily lysed. This subgroup of type 2A patients responds well to DDAVP for acute bleeding.


DDAVP was given to 22 patients with VWD and 10 with mild to moderate hemophilia A on two occasions, at least a month apart. Within-family consistency was seen when testing multiple members of five families. One test dose of DDAVP should suffice to predict a given patient’s response.


Intravenous DDAVP, 0.3 ug/kg, was given on each of four consecutive days to 22 patients with mild hemophilia A and 15 with type 1 VWD. The increases in levels of FVIII, VWF:Ag, VWF:RCo and plasminogen activator obtained after the second dose of DDAVP were about 30% less than those obtained after the first dose, but did not decrease further with the third and fourth doses. Patients with VWD tended to maintain day-to-day responsiveness better than those with hemophilia A. Prolonged BTs, however, improved after DDAVP about equally on each consecutive day of treatment. (The higher the baseline levels of FVIII and VWF:RCo, the more robust the response, and the more likely were daily doses able to keep plasma levels high enough.)


DDAVP infusion did not release tissue plasminogen activator (t-PA) in four patients with type 3 VWD. Seventeen patients with moderate VWD had increased baseline levels of t-PA measured immunologically (t-PA Ag). After DDAVP, further t-PA Ag was released. When t-PA was measured in a functional assay, the level was lower in moderate VWD than in normal persons. “The results suggest either that patients with VWD have a double defect in VWF and tissue plasminogen activator or that the primary deficiency of VWF influences the synthesis and/or release of t-PA by endothelial cells.”


In London in a ten-year period, 27 pa-
tients with type 1 or 2M VWD were treated with DDAVP for 35 surgical events. Intervals between DDAVP infusions (0.3 micrograms/kg) varied from 12 to 48 hours. Tranexamic acid was usually also used for mucosal surgery. Hemostasis was excellent in 91% of procedures; in only one patient with a rhinoplasty was hemostasis poor, requiring a third dose two days post-operatively.

FVIII-VWF concentrates were used for 68 surgical procedures in 26 patients with type 1 VWD, three with type 2A, three with type 2B and three with type 3. Hemostasis was excellent after 56 operations and moderate after six operations; in the latter, minor post-operative bleeding did not necessitate intervention. Hemostasis was poor with six operations, with rebleeding within the first two weeks necessitating further concentrate. Average pre-operative doses were 34 FVIII U/kg for dental procedures and 54 FVIII U/kg for other surgical procedures and deliveries. Average doses in the first 24 post-operative hours were 47 FVIII U/kg for major operations and 26-32 FVIII U/kg for lesser procedures. Similar doses were used on subsequent days. Treatment continued an average of 10 days for major operations, four for minor operations, six for ENT procedures, 7 for deliveries and one for dental procedures.

In 77 patients with type 1 VWD, “complete” responses were observed in 83% and no response in 4%. Patients with some abnormality of VWF multimeric pattern had less complete responses than those with normal patterns. Most patients with partial or no response had mutations in the A1-A3 domain. The best responses (but shortest T1/2) were seen with mutations in codons 1130 and 1205 in the D’-D3 domain.

DDAVP Nasal Spray


In 11 patients with mild hemophilia A and 11 with VWD, the effect of intravenous infusions of 0.3 ug DDAVP/kg was compared to that of intranasal spray of 150 ug to each nostril. In those with VWD, the rise in FVIII and VWD were greater with intravenous than with intranasal administration. Baseline BTs were prolonged in eight VWD patients and became normal in five of them after DDAVP by either route.


The effects of three intranasal doses (300, 450 and 500 ug) of DDAVP were compared in 5 patients with hemophilia A and 11 with VWD. There was no significant difference in response to the various dosages so the lower dose was recommended. (In VWD, the rise of FVIII was somewhat dose-related but the rise of VWF was not.) Five patients with VWD were followed for 24 hours, of these, two had increases of FVIII and VWD persisting for 8-12 hours; in the others, levels of these factors returned to baseline by 8 hours.


Test doses of 300 ug intranasal DDAVP were given to 39 patients with mild hemophilia A or VWD; 32 of these patients had good responses with correction of BTs and elevation of VWF to 50% and of FVIII to at least 30%. The responsive patients were
given the nasal spray to use at home, where they found they were able to achieve satisfactory hemostasis in 166 of 184 bleeding episodes.


A total of 2170 doses of intranasal DDAVP were used by 278 patients with VWD or mild hemophilia A in various centers. Patients rated efficacy as excellent or good in 95% of 384 bleeding episodes, 413 administrations for prophylaxis, and in all eight uses before dental or surgical procedures. When used for control of menorrhagia, efficacy was rated as excellent after 92% of 721 daily doses. Results were similar in the VWD and hemophilia groups. Adverse events were reported with 8% of the doses (172 doses), and consisted primarily of headaches and flushing, and, less commonly, dizziness or nausea. In two episodes, these symptoms were severe. In one episode, hyponatremia and edema occurred in a woman with a prior history of hyponatremia.


DDAVP vs. placebo nasal sprays were compared in women with inherited bleeding disorders; 24 women completed both arms of the cross-over design. The level of menorrhagia, as quantitated by PBAC scores, was lower, but not significantly different, on DDAVP vs. placebo.

DDAVP in type 2B VWD


Four patients with type 2B VWD (two of whom had intermittent thrombocytopenia) were treated with DDAVP intravenously. After infusion, platelet counts fell markedly (from 80-237,000/cu mm to 10-53,000 / cu mm) and platelets were aggregated on microscopy. Adsorption of VWF onto platelets was demonstrated. The authors recommended that DDAVP not be given to type 2B patients (but with the advent of the AIDS epidemic soon after this paper, they preferred to use DDAVP for type 2B patients over plasma products.)


A woman with type 2B VWD was treated with DDAVP for a cholecystectomy because she and her affected son had previously responded well to the agent (before being classified as type 2B) and the patient wanted to avoid the risk of viral transmission with blood products. After the first dose of DDAVP, her level of FVIII improved from 41% to 183%, the BT decreased from 15+ minutes to 7 minutes, and the platelet count fell from 262,000/cu mm to 183,000. The lowest platelet count seen, after her fifth and last dose of DDAVP, was 110,000. She had excellent hemostasis and no thrombosis. The authors concluded that DDAVP should not be ruled out categorically in type 2B VWD.


The authors review published reports

Two markers were studied to eluc-
date mechanisms of thrombocytopenia after DDAVP in four patients with type 2B VWD. One was plasma levels of platelet glyocalicin (a portion of GPIb, increased with platelet turnover) and the other was platelet surface expression of the alpha granule protein P-selectin (increased with platelet activation). At baseline, glyocalicin levels were normal except in one patient who, nevertheless, had a normal platelet count. Levels of platelet surface P-selectin were normal at baseline. DDAVP infusion resulted in decreased platelet counts but no increase in plasma glyocalicin levels or platelet expression of P-selectin. The acute thrombocytopenia after DDAVP infusion is not related to platelet activation or consumption.

DDAVP and hyponatremia


A man with moderate hemophilia A was treated with DDAVP in a dose of 0.5 ug/kg (higher than the usual dose of 0.3 ug/kg used nowadays) every 12 hours for five infusions. He developed hyponatremia with a plasma sodium of 124 nmol/l; his only symptom was a headache. The authors advised fluid restriction with DDAVP.


Four children, under age two years, developed hyponatremia less than 24 hrs after receiving DDAVP in the usual dose of 0.3 ug/kg; three had one dose and one had two doses 11 hours apart. Two patients were on intravenous fluids after minor surgical procedures. Three had grand mal seizures. The authors advised fluid restriction and avoidance of hyponatremic intravenous solutions.


Four patients with bleeding disorders were given DDAVP before surgery and afterwards at eight hour intervals. Three received intravenous fluids. Hyponatremia ensued. The two young children had seizures whereas the older patients had other symptoms of disturbance of the cerebral cortex. The authors warn against closely-repeated doses of DDAVP and use of intravenous fluids. Details of their cases are as follows:

<table>
<thead>
<tr>
<th>Age, yrs</th>
<th># of doses</th>
<th>Serum sodium (mEq/L)</th>
<th>Clinical result</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3</td>
<td>120</td>
<td>Seizure</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>121</td>
<td>Seizure</td>
</tr>
<tr>
<td>15</td>
<td>22</td>
<td>118</td>
<td>Obtundation</td>
</tr>
<tr>
<td>28</td>
<td>4</td>
<td>118</td>
<td>Agitation, confusion</td>
</tr>
</tbody>
</table>

An eight-year-old girl with VWD received one dose of DDAVP prior to tonsillectomy and a second dose 12 hours afterwards. In the post-operative period, she was given intravenous fluids and also drank water. She developed hyponatremia, with a seizure 27 hours after surgery. A 13-month-old boy with mild hemophilia received one dose of DDAVP for a circumcision and was on intravenous fluids and oral fluids thereafter. He developed hyponatremia and had a seizure 18 hours after the operation.


Hyponatremia (121 mEg/L) and lethargy developed after three daily intravenous doses of DDAVP at 0.3 ug/kg in a healthy woman, age 33, with moderate VWD who received DDAVP for sinus surgery. Neither her oral nor her intravenous fluid intake was excessive. She recovered with restriction of fluids to less than 500 ml/day.


A woman with VWD who had previously received many doses of DDAVP without difficulty was treated with DDAVP and ibuprofen (for pain) for a tooth extraction. The next day she was nauseated and dizzy. The following day she was found in a coma, with hyponatremia of 121 nmol/L. Ibuprofen is an antagonist of prostaglandin synthesis; renal prostaglandins antagonize the anti-diuretic effects of vasopressin. The two agents may have had additive anti-diuretic effects.

**DDAVP and myocardial infarct**


A 79-year-old man with mild hemophilia A and a 15 year history of angina was given DDAVP for iliofemoral bypass grafting. His second dose was 24 hours later, and eight hours after that he had an acute myocardial infarct. He then was treated with FVIII concentrate but some 9 days post-op, a third dose of DDAVP was given and a few hours later he suffered a fatal myocardial infarct. The timing of events suggests a relationship to DDAVP. (Commentators said that the myocardial infarct was not necessarily caused by DDAVP, given his pre-existing problems.)


A 47 year old man had multiple risk factors for myocardial infarct. He came from a family with early cardiac disease. He was a regular smoker and had elevated blood levels of cholesterol and triglycerides. He had donated plasma by plasmapheresis for his hemophilic son on 143 occasions, and on 88 of these, he had received DDAVP before the procedure to elevate his FVIII level. On the last occasion, he suffered a myocardial infarct 30 minutes after the end of the DDAVP infusion. The author states that there had been ten reports between 1985 and 1988 of myocardial infarcts following DDAVP use.

Mannucci PM, Carlsson S, Harris AS. Desmopressin, surgery and thrombosis. Thromb
DDAVP came to be used for persons who did not have bleeding disorders for surgical procedures associated with loss of large amounts of blood, to avoid the need for transfusion, especially during the AIDS epidemic. DDAVP greatly reduced blood loss but reports of myocardial infarcts and other thrombotic events frightened clinicians. The affected patients were often elderly and had other risk-factors.

In this meta-analysis of controlled clinical trials of DDAVP in surgery, 956 patients had received DDAVP and 877 placebo. There were a total of 57 thrombotic events, 33 in patients on DDAVP (3.4%) and 24 in patients on placebo (2.7%). Myocardial infarct occurred in 1.9% of patients on DDAVP and 1.4% of those on placebo.


In this meta-analysis, the use of DDAVP resulted in a small decrease in perioperative blood loss in cardiac surgery, but a 2.4-fold increase in myocardial infarction.

Plasma fractions
See also “cross-transfusion experiments”.


An early preparation of low-purity FVIII-VWF concentrate was used for surgery in three patients with severe VWD and one with moderate VWD, all with prolonged Ivy BTs. The concentrate raised the plasma FVIII levels consistently. Post-operative bleeding was associated with falls in FVIII levels. The BTs were unaffected or slightly but transiently improved. The authors concluded that correction of FVIII levels, but not correction of BTs, was important for hemostasis.


Previous studies in Sweden had shown that infusion of Cohn plasma fraction I, which contained FVIII-VWF, shortened the Duke BT. In this study in Norway of patients with VWD with prolonged BTs by both the Duke and Ivy methods, the Duke BT improved to near-normal in three of four patients infused with fraction I whereas the Ivy BT remained prolonged, or shortened only slightly, in five patients tested.


Three patients had major surgical operations under cover of Cohn plasma fraction I and normal whole blood. A patient with severe VWD continued to have prolonged Duke and Ivy BTs but had excellent hemostasis during surgery. Two patients with moderate VWD continued to have prolonged Ivy BTs but their Duke BTs were corrected; both had excellent hemostasis. The results show “that there is no correlation between the Ivy bleeding time and surgical hemostasis…” (Cross-transfusion experiments also are discussed.)

In two patients with severe VWD infused with cryoprecipitate, elevation of FVIII and VWF:Ag levels into the normal or borderline range persisted for 48-72 hours; levels of VWF:RCo reached the normal range but fell more swiftly. Correction of Ivy BTs lasted only 6-8 hours. FVIII-VWF concentrate infusion resulted in a lesser degree of correction of VWF:RCo and the BT than did infusion of cryoprecipitate.


Plasma Cohn fraction I and a fibrinolytic inhibitor (e.g. EACA) were used to provide hemostasis for 58 major surgical operations in 38 patients with VWD in Sweden. For severe VWD, a pre-operative dose of 30-40 FVIII U/kg was given; the patient’s FVIII level was checked to make sure it was about 50% and the Duke BT was checked to make sure it was normal. Another dose was given 4-5 hours post-operatively and again every 12 hours for the first 2-7 post-operative days to keep the Duke BT normal or only slightly prolonged and the FVIII level about 40%. Depending on the surgical procedure, infusions might continue every 24-48 hours beyond the 7th post-operative day. Lesser doses of fraction I were used in mild VWD. Hemostasis was regarded as excellent but whole blood transfusions were given during the operation in 12 instances, and afterwards (a single unit of blood) in six instances.


Cryoprecipitate was made from the plasma of a single designated donor repeatedly plasmapheresed, as part of an effort to avoid transmitting blood-borne viruses. A young man with type 1 VWD had a craniotomy under cover of such cryoprecipitate, in sufficient doses to maintain his VWF:RCo over 50% for two weeks. Three doses were given on the day of surgery and two on the first post-operative day; thereafter one dose (820 VWF:RCo units) was given per day.


After storage for 5 days, platelet concentrates retained 82% of their baseline VWF:RCo content but the proportion of smaller molecular weight multimer increased. (In some circumstances in VWD patients, platelet transfusions are advised; the stability of VWF at the usual room temperature of platelet storage might be questioned.)

FVIII-VWF concentrates.

Characteristics


Multimer analysis and levels of VWF:RCo and VWF:CB were compared for FVIII concentrates. In all products, levels of VWF:Ag were higher than levels of VWF:RCo or VWF:CB and were usually higher than FVIII. Retention of high molecular weight multimers in the concentrate correlated with higher levels of VWF:RCo and VWF:CB. (A good figure depicts multimer patterns of VWF:Ag from various concentrates available at the time.)
Multimer patterns of VWF from 13 FVIII-VWF concentrates were compared. None had the largest multimers seen in normal plasma but some had higher molecular weight multimers than others.

The old ("AHF High Purity") and the new ("Biostate®") FVIII-VWF concentrates made by CSL in Australia were compared to Humate P®. On average, there were about 1.8 VWF:RCo units per FVIII unit in AHF High Purity, about 2.4 VWF:RCo units per FVIII unit in Biostate® and about 2.6 VWF:RCo units per FVIII unit in Humate-P®. Biostate® appeared comparable to Humate-P® in content of large multimers and proportion of functional VWF. (A good figure depicts multimer distribution in the three concentrates.)

<table>
<thead>
<tr>
<th>Concentrate</th>
<th>Units of VWF: RCo per unit of VWF:Ag</th>
<th>Units of FVIII per unit of VWF: RCo</th>
<th>% of HMW multimers &gt;7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal plasma</td>
<td>“1”</td>
<td>“1”</td>
<td>“100”</td>
</tr>
<tr>
<td>Purified VWF concentrate</td>
<td>0.72</td>
<td>0.02</td>
<td>82</td>
</tr>
<tr>
<td>Humate-P</td>
<td>0.91</td>
<td>0.50</td>
<td>91</td>
</tr>
<tr>
<td>Inno-Brand</td>
<td>0.89</td>
<td>0.40</td>
<td>100</td>
</tr>
<tr>
<td>Koate DVI</td>
<td>0.48</td>
<td>0.85</td>
<td>61</td>
</tr>
<tr>
<td>8Y</td>
<td>0.29</td>
<td>1.23</td>
<td>52</td>
</tr>
<tr>
<td>Immune</td>
<td>0.15</td>
<td>6.00</td>
<td>15</td>
</tr>
</tbody>
</table>

Six concentrates available in Europe at the time were studied: Humate P® (Germany, ZLB Berhing), Immunate® (Austria, Baxter), Koate DVI® (USA, Bayer), 8Y® (England, BPL), Inno-Brand® (France, LFB) compared to normal plasma and to LFB’s purified plasma VWF. HMW multimers were most nearly normal in Humate-P® and Immune®, followed by Koate DVI®, then 8Y. Levels of VWF:Ag were similar to those of VWF:RCo in Humate-P® and Inno-Brand® (correlating with presence of HMW multimers) and, in both, were about twice as high as FVIII. The other three brands of FVIII-VWF concentrate had at least twice as much VWF:Ag as VWF:RCo, suggesting that much of the VWF:Ag was present as LMW multimers. Collagen binding activities are also described. Relative levels of VWF:RCo and FVIII and HMW multimers were as follows:


Twelve concentrates containing both VWF and FVIII had widely-varying levels of high-molecular-weight VWF multimers, and those levels correlated with levels of VWF:RCo and VWF:CB. The authors suggest
that concentrates with the highest levels are preferable for treatment of VWD.


The ratio of VWF:RCo to FVIII for this product has hovered around 1.0 for several years, in contrast to earlier reports.

**FVIII-VWF concentrates, use**


The responses of four patients with type 1 VWD, four with type A and two with type 3 VWD to infusions of Humate-P® are described (with excellent graphs). The Duke BT, FVIII, VWF:Ag and VWF:RCo corrected in all patients. The BT correction was the most transient. Multimers of all sizes were seen in patients’ plasma after infusion.


The concentrate provided effective hemostasis in 11 patients with VWD. In a 63 kg adult with type 3 VWD undergoing major surgery, 1000 FVIII units were infused daily for four days prior to surgery, three times on the day of surgery, twice daily for six days, and once daily for eight more days. Levels of FVIII were raised above 500% on the day of surgery and remained above 100% for the rest of the course of treatment. Levels of VWF:Ag peaked above 300% on the day of surgery and remained above 50% postoperatively. (Pictures of multimer patterns after infusion of Humate P® in three patients show rapid disappearance of large multimers.)

Federici AB, Mannucci PM. Optimizing therapy with factor VIII/von Willebrand factor concentrates in von Willebrand disease. Haemophilia 1998; 4 (suppl 3) 7-10 (Review)

“No FVIII/VWF concentrate had an intact multimeric structure similar to that of normal plasma or of cryoprecipitate; all FVIII/VWF concentrates were equally effective in attaining normal and sustained levels of FVIII:C post infusion although peak levels were more delayed in the concentrate devoid of FVIII, and no FVIII/VWF concentrate consistently normalized the BT in a sustained fashion. On the other hand, clinical hemostasis can be achieved in the management of bleeding episodes and of surgery for most of von Willebrand disease cases regardless of whether the BT is corrected; in the few rare cases with mucosal bleeding not controlled by FVIII/VWF concentrates, infusion of DDAVP or platelet concentrates can be administered in addition.”


During the years 1987-1997, 27 VWD patients were treated with DDAVP for 35 surgical events and 38 VWD patients were treated with concentrates for 68 elective surgical events. Most type 1 VWD patients and some type 2M patients received DDAVP. The drug was given every 12-48 hours, usually with tranexamic acid, depending on the type of surgery, for a maximum of six days. The efficacy was judged to be excellent in 91% of events. Factor VIII-VWF concentrates were used for ten major surgical operations. The median pre-operative dose was 54 FVIII U/kg and the median post-operative dose was 43 FVIII U/kg. Minor surgery doses were only slightly lower. Days of treatment for major surgery ranged from 4 to 14, median 10. Efficacy of concentrates for was judged to be
excellent in 82% of events. (Concentrate was used for patients whose VWD was more severe than those who received DDAVP.)


Humate-P® dosage was based on VWF:RCOF units from late 1991 to spring 1996 in Canada, during which time 97 patients were treated for 437 events including 73 surgical interventions. Clinical results were good or excellent in 97% of events. The median dosage for hemorrhages was 45-55 VWF:RCo U/kg per infusion. The dosage before surgical interventions was 55-70 VWF:RCo U/kg. The authors discuss the “contentious issue” of minimum dosage needed for hemostatic efficacy: some patients are treated successfully with doses lower than 20 VWF:RCo U/kg. The minimum effective dose is “unresolved”.


A high-purity FVIII-VWF concentrate (Alphanate®) was used in 81 patients with VWD. In pharmacokinetic studies in type 3 VWD, the post-infusion T ½ of FVIII was about 22 hours, of VWF:Ag was about 12 ½ hours, and of VWF:RCo was about 7 hours. Acute bleeding episodes were controlled with one or two infusions of 40 VWF:RCo units/kg in adults, 50 VWF:RCo U/kg in children, in 85% of instances. Patients with type 3 VWD typically required more infusions and higher doses. For invasive procedures and surgical operations, 60 VWF:RCo U/kg in adults, 75 U/kg in children, were given before the procedure and 40 units/kg for post-operative infusions (the number and frequency of which were not standardized). Prolonged BTs were corrected in 40% of patients. In only 3 of 71 procedures was there excessive bleeding. Two patients had thrombotic complications (thrombophlebitis in the arm of one man, deep vein thrombosis in the leg of another).


A survey of hemophilia centers around the world was undertaken to seek the incidence of thrombotic problems in the previous decade in patients with hemophilia A or VWD after treatment with concentrates. Two instances of DVT were reported in 14,125 patients with hemophilia A and seven instances (in addition to those in the paper above) in 1,268 patients with VWD. Characteristics of the latter patients included age 58 or older in five patients, joint replacement surgery in three patients and prolonged treatment for gastrointestinal hemorrhage or a target joint in four patients. A variety of concentrates had been used. Dr. Mannucci wonders whether excessively high FVIII levels, resulting from use of FVIII-VWF concentrate, provoked thrombosis.


Four patients with VWD treated in the United Kingdom with FVIII-VWF concentrate (Humate-P®) for invasive or surgical procedures developed deep-vein thrombosis; one had a pulmonary embolus. None had received unusually high doses or prolonged treatment but all had underlying risk factors.
Deficiencies of AT III, protein S and C were ruled out in all patients, and in three, tests for factor V Leiden, prothrombin gene mutation and antiphospholipid antibodies were carried out and were negative. The authors wonder whether a higher-than-normal level of FVIII induced by therapy might be dangerous.


Treatment regimens of Humate-P® were based on dosage with VWF:RCo international units (IU) in 26 patients who had 43 surgical or invasive procedures. On average, there was 2.2 times as much VWF:RCo as FVIII in the lots used in the study. The mean initial dose for major surgery was 61.2 VWF:RCo U/kg, for minor surgery was 49.8 U/kg, for dental extractions was 35.2 U/kg and for invasive procedures was 43.6 U/kg. The mean daily dose after major surgery was 39.3 U/kg for major surgery and 28.7 U/kg for minor surgery. The duration of treatment averaged 9.7 days for major surgery, 4.2 for minor surgery, 1.6 for dental surgery and 2.7 for invasive procedures. FVIII was measured daily. The mean level after major surgery was 104% and the highest level was 185%. One patient who had multiple dental extractions had a hemorrhage from the gums 3 days after surgery and required extra doses of concentrate. In all others, hemostatic control was effective.

Humate-P® dosage was based on VWF:RCo international units. In 39 VWD patients of a variety of types having a total of 42 surgical procedures, the median initial dose was 82.3 VWF:RCo IU/kg and the median subsequent dose was 52.8 U/kg per infusion for a median duration of three days. Dosage tended to be higher with major procedures than in seven subjects; the mean peak level of VWF:RCo was 183% and of FVIII was 122%. Levels later in the course were not quoted. Efficacy was rated as excellent or good in 100% of events.

(In the above recent reports, the total number of units given pre-operatively and in the first two days, about 220 VWF:RCo units/kg, may be compared to the approximate 190 FVIII units/kg I give for surgical operations in severe hemophilia A during the same time period. Both dosage levels, for VWF and for hemophilia A, are generous and do not establish the necessary minimum.)


Concentrate dosage for surgical operations in 28 subjects with types 1, 2A, 2M or 3 VWD was adjusted according to results of prior pharmacokinetic studies. For major surgery, median post-operative levels of VWF:RCo were 46.1% on days 0-2 post-op days and 43.9% on days 3-6 post-op. Hemostasis was excellent. (Lower doses worked.)


A plasma-derived concentrate with equal amounts of VWF and FVIII and no albu-
min was used successfully to halt acute hemorrhages in VWD. Nineteen severely-affected patients used the concentrate prophylactically, at mean doses of 27.4 IU/kg, at a mean frequency of 1.9 infusions/week, with a drop in the frequency of hemorrhages from a mean of 4.5/month to a mean of 1.4/month.


In a multi-center prospective trial completed by 11 subjects (six type 2A VWD with VWF:RCo < 20% who were unresponsive to DDAVP, and five with type 3 VWD) various dose levels were tried. Reasons for prophylaxis were epistaxis in 6 patients, GI bleeding in three and joint bleeding in two; none of the initial group enrolled for control of heavy menses. Seven patients were escalated from the first dose level (50 IU VWF:RCo/kg once a week) to the second dose level (same dose twice a week), and one patient was escalated to the third dose level (same dose three times a week). One patient with GI bleeding had to be advanced to treatment every other day. This is a very welcome trial that actually compares dosage levels! It is ongoing and still accruing patients.

Purified VWF Concentrates


A plasma-derived concentrate, made in France, had more than ten times as much VWF as FVIII. It provided good hemostasis in patients with various types of VWD. In a type 3 patient, the half-life of VWF:Ag was 20.6 hours, of VWF:RCo was 17.8 hours, and of FVIII was 74 hours.


Solvent-detergent treated plasma-derived VWF concentrate, very low in FVIII, had been used in France since 1989 for treatment of 75 patients with VWD on 99 occasions. Spontaneous bleeding (other than gastrointestinal bleeding) usually responded to infusions of about 40-47 VWF:RCo U/kg. Surgical operations were managed as follows: (1) patients with baseline levels of FVIII of more than 20% were given one pre-operative infusion of 51-55 U/kg an hour before surgery, which raised plasma FVIII levels into the low-normal range and VWF:RCo into the average-normal range. (2) patients with baseline levels of FVIII of less than 20% were given two pre-operative doses, 12 or 24 hours apart, or, when the situation was urgent, were given infusions of FVIII concentrate in addition to VWF concentrate pre-operatively. During the post-operative period, VWF concentrate was given every 12-24 hours in a dose of 30-35 VWF:RCo U/kg which maintained the plasma FVIII level between 118-138% (maximum observation 180%) and the VWF:RCo between 76-107%. Patients received 1-11 infusions over 1-5 days for minor surgery and 6-16 infusions over 6-16 days for major operations. Patients with type 2N were given infusions of VWF concentrate for surgical operations but received a VWF concentrate plus FVIII concentrate for acute hemorrhages.

Borel-Derlon A, Federici AB….Mannucci PM et al. Treatment of severe von Willebrand disease with a high-purity von Willebrand factor concentrate (Wilfactin®): a prospective study of 50 pa-
The above plasma-derived VWF concentrate with low FVIII content was used in doses of 50-60 VWF:RCo IU/kg to treat 139 spontaneous bleeding episodes in 50 patients with clinically-severe VWD (loosely defined), the group included types 1, 2 and 3; 72% of subjects had <10% VWF:RCo and 46% had <20% FVIII). The outcome was excellent or good in 89% of episodes. The same concentrate was used in 44 of the patients for 108 invasive or surgical procedures with excellent results. Doses were 50-60 IU/kg 12-24 hours before surgery and again an hour before surgery and thereafter twice daily as needed to maintain target levels of 40-60 % VWF:RCo for about 10 days. For emergency procedures, the initial dose of this concentrate was accompanied by a dose of FVIII concentrate. Although factor VIII levels rose (from endogenous production) in multiply-treated surgical patients, no thrombosis occurred. These doses were generous, and the actual VWF:RCo levels maintained post-operatively, read from a graph, tended to be higher than the targeted levels. Use of this concentrate avoids high plasma levels of FVIII from exogenous sources.

**Recombinant VWF concentrate**


Dogs with severe VWD were given a recombinant VWF concentrate containing multimers of all sizes which was hemostatically effective. The half-life of VWF:Ag was about 22 hours.


A recombinant VWF had a homogeneous and intact VWF multimer distribution because it was not exposed to ADAMTS13 during manufacturing. For this trial, it was combined at a fixed ratio (1.3:1) with recombinant FVIII. Subjects had type 3 or severe type 1 or 2A VWD. Crossover pharmacokinetic studies compared the effects of this recombinant combination with that of a plasma-derived (pd) VWF-FVIII. The mean clearance of the recombinant mixture was 2.2 mL/kg/hr, lower than the 3.4 mL/kg/hr of the pd product. The T1/2 of VWF:Ag was 25.5 hrs with the recombinant and 17.9 hrs with the pd products. There was a greater secondary rise in FVIII after the recombinant product; higher levels at 72 and 96 hrs post-infusion were attributed to better stabilization of endogenously-produced FVIII by rVWF vs. pdVWF, perhaps because of a longer T 1/2 of rVWF or larger multimers.


The recombinant VWF (rVWF) concentrate described above was given with and without rFVIII in patients with type 3 or severe type 1 or 2A VWD. The terminal half-life of VWF:RCo was 21.9 hours for rVWF alone and 19.6 hours for rVWF combined with rFVIII. In subjects receiving rVWF alone, their (endogenously-produced) median FVIII levels rose above 40% by 6 hrs and peaked at 86% by 24 hours after infusion. Treatment of acute hemorrhages was with the rVWF-rFVIII product primarily and was successful. The product to be marketed by Baxalta as “Vonvendi®” is the concentrate of rVWF alone, NOT with rFVIII.
Prophylaxis in VWD


A plasma-derived concentrate with equal amounts of VWF and FVIII and no albumin was used successfully to halt acute hemorrhages in VWD. Nineteen severely-affected patients used the concentrate prophylactically, at mean doses of 27.4 IU/kg, at a mean frequency of 1.9 infusions/week, with a drop in the frequency of hemorrhages from a mean of 4.5/month to a mean of 1.4/month.


Patients with severe VWD, unresponsive to DDAVP, with frequent bleeding, were started on weekly doses of 50 IU VWF:RCo/kg and escalated as needed to two, then three such infusions/week. Four subjects with epistaxis only were able to remain on one infusion/week, three subjects with various types of bleeding escalated to two infusions/week and three to three infusions per week, and one more subject, with GI bleeding, escalated to every-other-day infusions. Bleeding logs of ten subjects were evaluable; annualized bleed rates dropped from a median of 25.0 to a median of 6.1. No specific brand of concentrate was required by this international study.


Prophylaxis in VWD is not new but has not yet been extensively documented; it is growing in popularity. Candidates for long-term prophylaxis are patients with any VWD type whose hemorrhages require intense on-demand therapy with VWF concentrates. The decision for prophylaxis is different from that in hemophilia. In severe hemophilia, “primary” prophylaxis is started at an early age. In VWD, the pattern of severe, frequent bleeding is first established and then “secondary” prophylaxis is started. Secondary long-term prophylaxis always requires the use of VWF concentrates (as opposed to DDAVP, even if the patient has some response to DDAVP.) The effective dosage and frequency of administration are being studied.