

# VON WILLEBRAND DISEASE

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**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 large multimeric glycoprotein made in endothelial cells and secreted into the plasma, and also made in megakaryocytes and present in platelets. VWF has two functions, as follows: (1) it attaches to subendothelial collagen and to platelets, promoting formation of 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; bleeding from small skin cuts and from lesions in the mucosa or gastrointestinal tract; menorrhagia and excessive bleeding after trauma, surgical operations or childbirth. Patients with severe deficiencies of VWF may bleed into joints or muscles.

The **prevalence** of symptomatic cases, requiring occasional transfusion treatment, is about one in 5-10,000 persons. The prevalence of severe VWD is highest in Sweden (one case per 200,000 inhabitants) and is also high in countries neighboring Sweden and in countries where consanguineous marriages are commonplace.

The pathogenesis of VWD and the nature of the relationship between FVIII and VWF were not elucidated until recent years. As comprehension improved, terminology changed. Standard terms, approved by the International Society on Thrombosis and Hemostasis, are used in this publication (Table 2). Older terms, when needed, are in quotation marks. The author's comments are in *italics*.

## **History**

In 1926, Dr. Erik von Willebrand of Helsinki published, in Swedish, 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 is 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 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 but with negative capillary fragility tests. 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 but was probably also co-incidental. In VWD, bleeding from vascular lesions may be excessive, calling attention to the presence of such lesions. Reports of capillary tortuosity, seen in the nail-beds, have been published from time to time, including a large series in 2000. This phenomenon has not yet been investigated thoroughly.

The saga of gradual enlightenment on the nature of VWD is described below because classic scientific papers that are still often cited contain allusions and nomenclature that can be understood only in the historical context (Table 1).

In the 1950's, with development of an **assay for FVIII**, hemophilia A could be defined clearly. The inheritance was obviously sex-linked. All affected males in a given family had 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. After plasma transfusion, the FVIII level remained elevated much longer in VWD than in hemophilia A. Plasma from patients with severe hemophilia A was transfused into patients with severe VWD, as an experiment. 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.

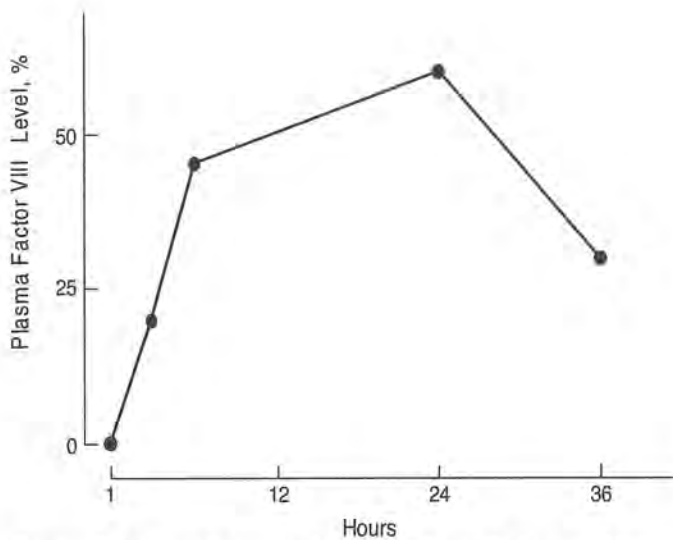
**Table 1. The development of tests is related to the evolving understanding of VWD.**

Years	Test	Comment
1910-1920's	Clinical observation	VWD, described in 1926, was recognized as different from hemophilia in its bleeding pattern (mucosal) and inheritance (autosomal).
	Bleeding time	The BT was prolonged in VWD but not in hemophilia.
1950's	Factor VIII assay	FVIII levels in affected males with hemophilia A in a given family were similar. FVIII levels in VWD also could be deficient but varied from one affected person to another within a family.
	Cross-transfusion experiments	When plasma from a patient with severe hemophilia A was transfused into a patient with severe VWD, the FVIII rose slowly and was elevated for days. When plasma from a patient with severe VWD was transfused into a patient with hemophilia A, his FVIII level was unchanged.
1970's	Ristocetin-induced platelet aggregation and ristocetin cofactor	Ristocetin aggregated platelets in platelet-rich plasma from normal persons and patients with hemophilia A but not from patients with VWD. A quantitative functional test (ristocetin cofactor, VWF:RCo) was designed.
	VWF measured immunologically	Antibodies raised in rabbits against semi-purified FVIII reacted with an antigen ("FVIII:Ag" = VWF:Ag) in plasma from patients with hemophilia A that was absent in plasma from patients with severe VWD.  FVIII and VWF were shown to be separate entities.
1980's	Multimer analysis	Immuno-electrophoresis displayed the size distribution of VWF multimers. Different patterns, consistent within families, led to classification of VWD types.
	Gene cloning, sequencing	The genes for FVIII and for VWF were cloned; the proteins were sequenced.
1990's	Collagen binding	Another functional test, VWF binding to collagen (VWF:CB), was designed.
	VWF-FVIII binding	A few patients with low FVIII, first thought to have mild hemophilia A, were shown to have defective binding of VWF to FVIII (VWF:FVIII:B), VWD type 2N
	Mutations defined, VWF expressed from cloned genes	Specific functional defects in VWF were linked to specific sites on the protein and on the gene. Mutant VWF was expressed from cloned mutant genes to determine how it was dysfunctional, shedding light on normal function.

Throughout the 1960's the relationship between FVIII and the plasma factor (VWF) deficient in VWD remained puzzling. Discoveries in the 1970's were helpful. **Ristocetin**, an antibiotic, was withdrawn from clinical use because it sometimes caused thrombocytopenia. It proved to be a useful reagent. Ristocetin stimulated the aggregation of platelets suspended in plasma (platelet-rich plasma) 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 platelet-rich plasma from a person with 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)**, evolved.

**Immunologic tests** helped clarify matters. When rabbits were injected with semi-purified FVIII (which included VWF, unrecognized at the time), they developed antibodies that recognized a substance in the plasma of all persons with hemophilia A. That substance was called "factor VIII related antigen" or FVIII:Ag (now known to be **VWF:Ag**). Further studies showed that the so-called "factor VIII



**Figure 1. Cross-transfusion. FVIII levels in a patient with severe VWD after infusion of plasma from a patient with hemophilia A in a hypothetical experiment, based on data from actual experiments.**

molecule” was composed of two entities, FVIII and VWF, which could dissociate. VWF was found to be a large **multimer** composed of repeating subunits. Immuno-electrophoresis displayed multimers according to size (Figure 2).

**In 1985, the VWF gene was cloned.** The gene and plasma protein were sequenced. Structure-function relationships could be studied.

**VWD was obviously heterogeneous.** Results of the newly-developed tests formed patterns that differed from one affected family to another. The first official **classification of VWD types, in 1984**, was based on multimer patterns (Table 3). Roman numerals were used. In “type I” VWD, multimers of all sizes were present, but in decreased concentration. In “type II” VWD, the largest (high-molecular-weight, HMW) multimers were absent. In “type IIA” VWD, no HMW multimers appeared after DDAVP stimulation, and, there was little or no aggregation of platelet-rich plasma with ristocetin. In “type IIB” VWD, HMW multimers did appear after DDAVP stimulation, and, platelet-rich plasma aggregated with lower concentrations of ristocetin than normal, that is, it hyper-aggregated. In “type III” (severe) VWD all multimers were absent and platelet-rich plasma did not aggregate with ristocetin.

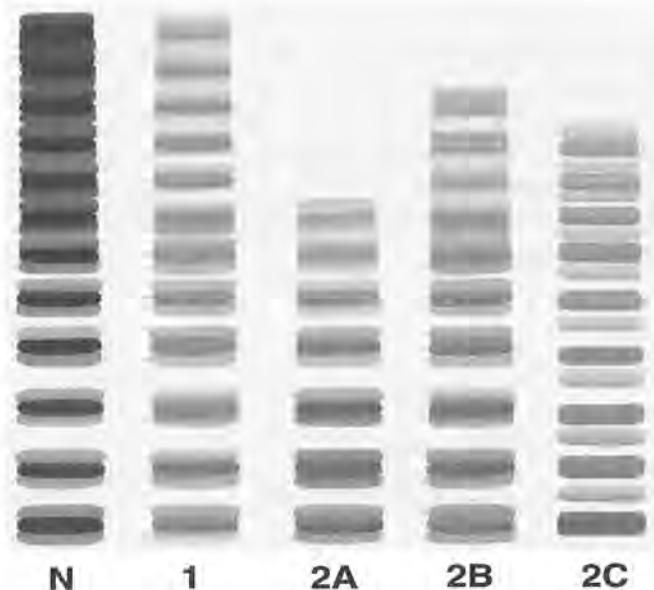
Families were soon found who did not fit into that classification. A slightly **revised classification, in 1994**, defined VWD types, using Arabic numerals, according to their presumed pathogenesis. Types 1 and 3 were quantitative; type 2 was qualitative. All VWD was believed to be related to abnormalities of the VWF gene. Subsequent studies showed that some patients who appeared to have type 1 VWD

had no VWF gene mutation. Such a mutation is no longer demanded for the diagnosis of VWD (see section on Type 1 VWD).

### VWF molecule

**VWF is synthesized in endothelial cells and megakaryocytes**, first as a precursor polypeptide consisting of a signal peptide of 22 amino acids, a propeptide (formerly known as “von Willebrand antigen II”, “VWAg II”) of 741 amino acids and a subunit of 2050 amino acids. Repeated domains in the subunit are illustrated in Figure 3. The mature subunit is extensively glycosylated,

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.)



**Figure 2. Multimers.** This drawing represents multimer distribution in various types of VWD. The largest multimers are on top. Note the fainter satellite bands around each major band. Type 2C (“IIC”) VWD is now classified under type 2A, but is illustrated as an example of abnormal multimer band structure. A satellite band appears half-way between each major

**Table 2. Official abbreviated terms as designated by the International Society on Thrombosis and Hemostasis and the old or informal abbreviated terms used for factor VIII and von Willebrand factor.**

Type of Test	Factor VIII		Von Willebrand factor	
	Official	Old or informal	Official	Old or informal
Immunological (total amount, functional or not)	FVIII:Ag	FVIII:CAg	VWF:Ag	FVIII:Ag AHF:Ag
Functional (functional assay)	FVIII	FVIII:C, AHF, AHG (anti-hemophilic factor or globulin)	VWF:RCo (ristocetin cofactor) VWF:CB (collagen binding) VWF:FVIII B (factor VIII binding)	FVIII:RCO and others VWF:CBA

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. **HMW VWF multimers bind to GPIb far better than do smaller ones.** 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. **Binding of VWF to FVIII is not dependent on multimer size.**

VWF circulates as elongated or coiled multimers. Upon stimulation, it uncoils into a long string, exposing many GP Ib binding sites.

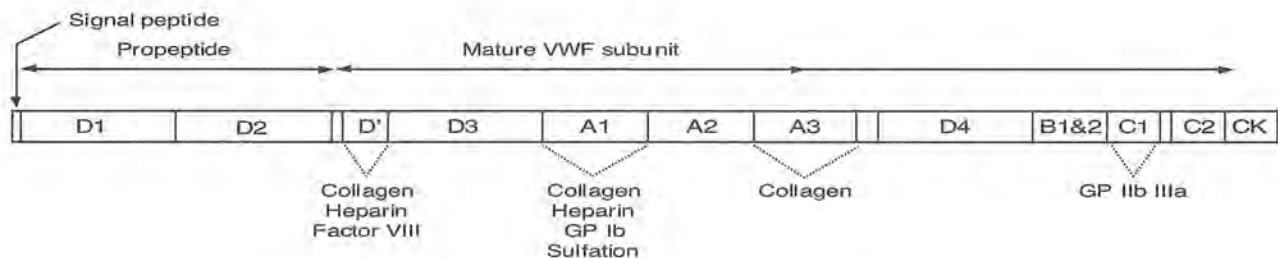
### VWF gene

The gene for VWF is at the tip of the short arm of chromosome 12. It is exceptionally large, with 52 exons and about 178 kilobases. A signal peptide and propeptide are encoded by about 80 kilobases and the mature subunit by the remainder. There is a pattern of repeated homologous sequence domains (Figure 3). A partial pseudogene is located on chromosome 22.

### 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 high levels of thyroid hormones as in hyperthyroidism and (5) with high levels of estrogen and progesterone as in pregnancy.

Levels also are related to **blood group and race**. Levels are higher in persons with blood groups A and B compared to blood group O. (Group O is associated with a relatively low level of protein glycosylation; VWF is less glycosylated in group O persons. Levels of the protease ADAMTS-13, which cleaves VWF, are higher in group O persons.) Levels of VWF are higher in persons of black African descent than in Caucasians. The normal range for black Africans who have non-O blood groups barely overlaps with that of Caucasians with group O. Other blood groups have lesser degrees of association. Normal inherited variations in the VWF gene, that is, **single nucleotide polymorphisms**, also influence levels of VWF, in part by affecting susceptibility to proteolysis.



**Figure 3. The VWF gene. VWF within the cell consists of a signal peptide, a propeptide and a mature subunit with repeating domains. There are three collagen binding sites at D', A1 and A3 domains. Factor VIII binds at the D' domain. Platelet GP Ib binds at the A1 domain. Platelet GP IIB/IIIa binds at the C1 domain.**

**Table 3. The 1984 and 1994 classifications of von Willebrand disease subtypes by the International Society on Thrombosis and Hemostasis.**

1984 term	1984 definition	1994 term	1994 definition	Genetics, Comment
Type I	Plasma contains multimers of all sizes; quantity decreased	Type 1	Partial quantitative deficiency of VWF	Dominant with variable expression; phenotype influenced by multiple genes
Type II	Largest multimers absent from plasma	Type 2	Qualitative defect of VWF	(subtypes below)
Type IIA	Largest multimers absent from platelets and plasma, even after stimulation with DDAVP	Type 2A	Decreased platelet-dependent function with absence of largest multimers	Dominant. With some mutations, large multimers do not form. With others, VWF is vulnerable to rapid proteolysis.
Type IIB	Largest multimers present in platelets; they appear in plasma after stimulation with DDAVP	Type 2B	Increased VWF affinity for platelet GPIb	Dominant. May be associated with thrombocytopenia, especially after DDAVP
		Type 2M	Decreased platelet-dependent function, with presence of largest multimers	Dominant. In the "Vicenza" variant, extra-large multimers are present, perhaps as a response to rapid proteolysis of the VWF molecule..
		Type 2N	Decreased VWF affinity for FVIII	Recessive. Often mistaken for mild-moderate hemophilia A.
Type III	No multimers (severe VWD)	Type 3	Virtually complete deficiency of VWF	Recessive: homozygous or doubly heterozygous
Platelet-type VWD	Largest multimers absent from plasma	pseudo-VWD	Not a defect of VWF, not to be considered a form of VWD	Dominant. A platelet disorder: increased affinity of platelet GPIb for VWF. Thrombocytopenia may be present.

### Type 1 VWD

The most common type of VWD is mild to moderate quantitative deficiency of VWF. Levels of FVIII, VWF:Ag and VWF:RCo are decreased to the same extent. Levels of multimers of all sizes are decreased to the same extent. Gene mutations, when found, are scattered in type and location and are typically dominant with variable penetrance. One person with a given genotype may be asymptomatic and have normal laboratory tests while another has mild to moderate symptoms and laboratory tests in the abnormal range. A few mutations in the D3 domain cause highly-penetrant dominant-negative mutations in which the mutant VWF retained intracellularly impedes the secretion of normal VWF. A few patients with type 1 VWD are heterozygous for a "null" muta-

tion, which codes for no production of VWF, and which, in homozygotes, causes type 3, severe VWD. (Conversely, only a small minority of heterozygotes with a null mutation have a VWD phenotype.)

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 (where available) are similar and are definitely below the normal range, and when the BT is prolonged. (Table 6.) Ristocetin induced platelet aggregation, RIPA, usually is normal or mildly reduced. Diagnosis is difficult in persons with mildly-deficient or borderline test results. There is substantial assay error in most of the tests used to diagnose VWD. There is some day-to-day variation in factor levels within a given person.



**Table 4. 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 that were defined as the mean minus two standard deviations.**

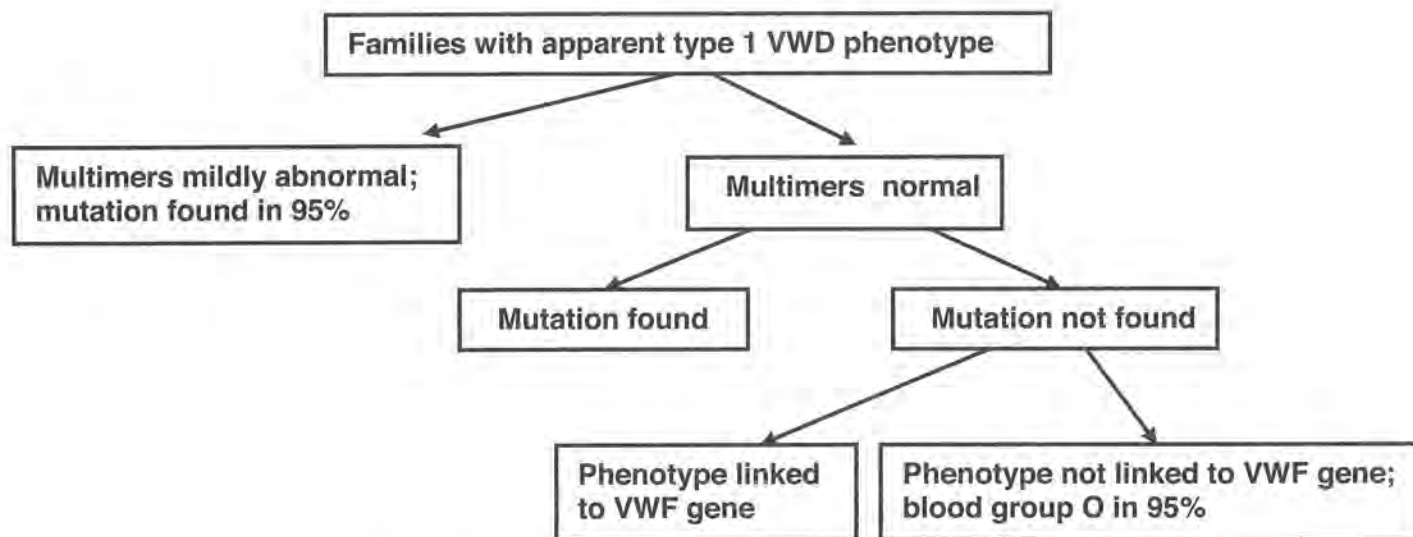
Blood group	n	VWF:Ag, mean, %	Range, $\pm 2$ SD
O	456	74.6	35.6-157.0
A	340	105.9	48.0-233.9
B	196	116.9	56.8-241.0
AB	109	123.3	63.8-238.2

**Table 5. 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.)**

Race & ABO Blood group	VWF:Ag %	VWF:RCo %	FVIII %
White, O	84	80	90
Black, O	104	80	100
White, non-O	113	110	109
Black, non-O	140	112	132

**Table 6. Typical levels of FVIII, VWF:Ag, VWF:RCo and VWF:CB in normal subjects, in severe hemophilia A and in various types of VWD. In normal persons and in type 1 VWD, levels are similar. Persons with hemophilia A retain the ability to make VWF so tests reflecting VWF are normal.**

Diagnosis	FVIII, %	VWF:Ag, %	VWF:RCo, %	VWF:CB	Comments
Normal subject	e.g. 50%	@50	@50	@ 50	Levels in a given person are similar, within assay error
Normal subject	e.g. 100%	@ 100%	@100	@ 100	
Hemophilia A	<1 %	e.g. 100 %	e.g. 100	e.g. 100	Ability to make VWF is unaffected; VWF is normal
Type 1 VWD	e.g. 10%	@ 10%	@ 10	@ 10	Levels in a given person are similar, within assay error
Type 1 VWD	e.g. 30%	@ 30%	@ 30	@30	
Type 3 VWD	Very Low	Undetectable	Undetectable	Undetectable	Small amounts of FVIII may circulate alone
Type 2A VWD	Mildly-low to low-normal	Mildly-low to low-normal	Definitely low	Definitely low	VWF function is reduced much more than quantity; use RIPA to distinguish type 2A from type 2B
Type 2B VWD	Mildly-low to low-normal	Mildly-low to low-normal	Definitely low	Definitely low	
Type 2 M VWD	Mildly-low to low-normal	Mildly-low to low-normal	Definitely low	Mildly-low, but higher than FVIII:RCo	VWF function is reduced much more than quantity, but collagen-binding is preserved better than in type 2A or 2B.
Type 2 N VWD	Definitely low	Mildly-low to normal	Mildly-low to normal	Mildly-low to normal	Diagnosis requires specific test of VWF:FVIII B
Pseudo-VWD	Mildly-low to normal	Mildly-low to normal	Definitely low	(no data)	Enhanced RIPA as in type 2B.



**Figure 4. Heterogeneity in patients with previously-diagnosed apparent type 1 VWD enrolled in European Union study. (Derived from data in Peake & Goodeve, *J Thromb Haemost* 2007, 5, suppl 1, 7-11.)**

Fears arose that type 1 VWD was over-diagnosed, based partly on the fact that blood group O is more prevalent in patients diagnosed with type 1 VWD than in the general population, and in part on the large number of patients with quite borderline test results who had been told they had type 1 VWD. In recent years, multicenter studies were undertaken of large numbers of patients who had been diagnosed with familial type 1 VWD in reputable hemophilia centers. In the largest study, in the European Union (Figure 4), a highly-sensitive test revealed subtle abnormalities in multimers in about a third of families. These patients tended to have lower levels of VWF than the group as a whole, and VWF gene mutations could be found in 95% of them. These patients might be classified as having type 2 VWD by a highly-expert laboratory. In contrast, VWF gene mutations could be found in only half the families with normal multimer structure. In those families with normal multimers in whom no gene mutation was detected, the phenotype of VWD was, nevertheless, linked to inheritance of the same VWF gene in about a third of instances, leading to suspicion that their condition had some genetic cause within the VWF gene or linked to it. Of the remaining two-thirds of families with normal multimers, no mutations and no linkage to inheritance of the same VWF gene, blood group O was found in 95%. The phenotype in these families may be caused by one or more genetic determinants that tend to depress the VWF level.

A mild bleeding tendency may be accentuated by defects unrelated to VWF, such as minor platelet abnormalities. The family history may be positive, for such defects may run in the family. A low VWF level may be a risk factor for excessive

bleeding, whether or not a VWF gene mutation is present.

In clinical practice, without genotyping, it is not possible to distinguish persons with apparent mild VWD who do have a VWF gene mutation from those who do not, or those with and those without subtle multimer changes. Thus, the diagnosis of mild type 1 VWD is based on clinical evaluation and easily-available laboratory tests and, in borderline cases, often is ambiguous.

### **Type 2A VWD**

Typical type 2A VWD is caused by dominant missense mutations, usually in the A2 domain, and occasionally in the A1 domain of the VWF gene. Penetrance is high. Some mutations impede multimer formation; no HMW multimers ever are formed. With other mutations, HMW multimers form but are vulnerable to rapid proteolysis in circulation and only low molecular weight (LMW) multimers remain.

Absence of HMW multimers greatly reduces VWF binding to platelet GPIIb. The depressed functional activity 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, as reflected by the VWF:Ag measurement, is only mildly depressed, and, therefore, is higher than VWF:RCo or VWF:CB. LMW 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, as in type 2A VWD: 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, however, 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 the RIPA test.

Patients with enhanced ristocetin-induced platelet aggregation, but with multimers of all sizes, formerly called "New York" or "Malmö" variants, now are included under type 2B.

## **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 multimer distribution and Type 2M designation. The responsible mutations are in the A1 domain. Certain patients, originally described in the Vicenza region of Italy, circulate multimers larger than any seen in normal plasma (possibly in response to rapid lysis.). Mutations responsible for the Vicenza phenotype are in the D3 domain.

## **Type 2N**

Type 2N VWD ("N" for "Normandy", the birthplace 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, but have normal, low-normal, or mildly depressed levels of VWF:Ag and VWF:RCo. Heterozygotes have normal levels of FVIII but can be demonstrated to have decreased VWF-FVIII binding with sensitive tests.

## **Other type 2 defects**

Occasional families have test results that do not fit cleanly into one of the major types defined in 1984 or 1994. Some patients originally classified as type 1, who have multimers of all sizes but a relatively greater deficit of HMW multimers than of LMW ones, are now included under the type 2A designation. Patients with unusual multimer band patterns were combined, in 1994, under type 2A; they include the former "type IIC" with mutations in the D2 domain, the former "type IID" with mutations in the CK domain, the former "type IIE" with mutations in the D3 domain, and others.

## **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. Certain mutations are especially recurrent in Scandinavia, where type 3 is most prevalent. Levels of 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 type 3 VWD patients. Homozygosity for large deletions 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.

## **Pseudo-VWD**

A gain-of-function mutation in the gene for platelet 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. 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. Patient platelets aggregate on addition of cryoprecipitate (which contains normal VWF), but some aggregation also may occur in type 2B VWD, making distinction difficult at times. Bleeding is treated with platelet transfusions.

## **Acquired VWD**

A VWD syndrome may appear in patients with hypothyroidism, autoimmune disorders, lymphoma, macroglobulinemia or other similar conditions. VWD may appear months to years before the

underlying disorder.

It may be difficult to demonstrate that the VWD is acquired and not congenital. Attempts to demonstrate an antibody to VWF are successful only in a minority of instances. Plasma levels of the VWF propeptide are typically higher than normal. Propeptide levels are assayed in reference laboratories.

A VWD syndrome, with a type 2A phenotype, has been described in persons with aortic stenosis, due, presumably, to consumption of HMW multimers at the valve. Excessive bleeding from gastrointestinal angiodysplasia is reported commonly in older patients with aortic stenosis. The acquired VWD, including the gastrointestinal bleeding, disappears after valve replacement.

### VWD and atherosclerosis

Pigs with type 3 VWD have less atherosclerosis than is seen in normal pigs, even when they are fed very high cholesterol diets from an early age. Humans with type 3 VWD appear to accumulate atherosclerotic lesions at or near the same rate as do normal persons.

### 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. 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.

**Menorrhagia** may be overlooked because a patient may not realize that her menses are atypical and because quantification of menstrual blood loss is difficult. A "pictorial blood loss assessment chart", "PBAC", was developed recently. It 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. Women with type 1 VWD who still have subnormal levels of VWF at term often are given DDAVP at delivery. Women with types 2 and 3 VWD may be given FVIII-VWF concentrates. Pregnancy-induced elevations of FVIII and VWF are lost within a few days of delivery. Late postpartum hemorrhages may ensue.

### Diagnostic 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. More objective and sensitive equivalents have been sought. In the 1960's and 1970's, the adhesion of the patient's platelets to glass beads was measured.

Recently, automatic platelet function analyzers, predominantly the **PFA-100®** made by Dade International, have become popular. Anti-coagulated fresh whole blood passes at a high shear-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 much 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 5). 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 the decreasing optical density. The more dilute the plasma, the longer the time to aggregation. A standard curve, plotted with normal reference plasma, relates dilution to aggregation time (Figure 5). The aggregation times of patient plasma are compared to the standard curve to quantitate von Willebrand factor activity as VWF:RC<sub>o</sub>. VWF:RC<sub>o</sub> levels are low in all types of VWD, except 2N. The test can be performed on shipped samples of plasma because the plasma can be frozen and thawed. The normal platelets can be fixed with formalin and either frozen or freeze-dried.

In **ristocetin induced platelet aggregation** (RIPA), there are two reagents, as follows: (1) **fresh** platelet-rich plasma and (2) ristocetin at two different

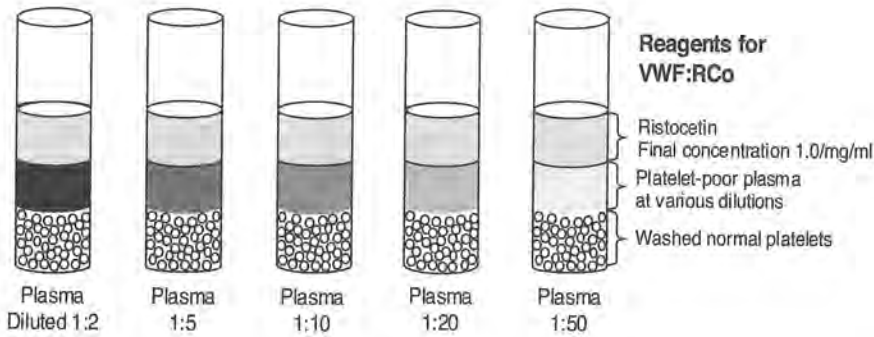


Figure 5. Tests using ristocetin.

*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.*

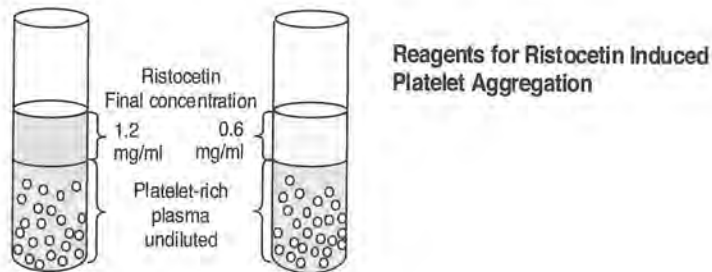
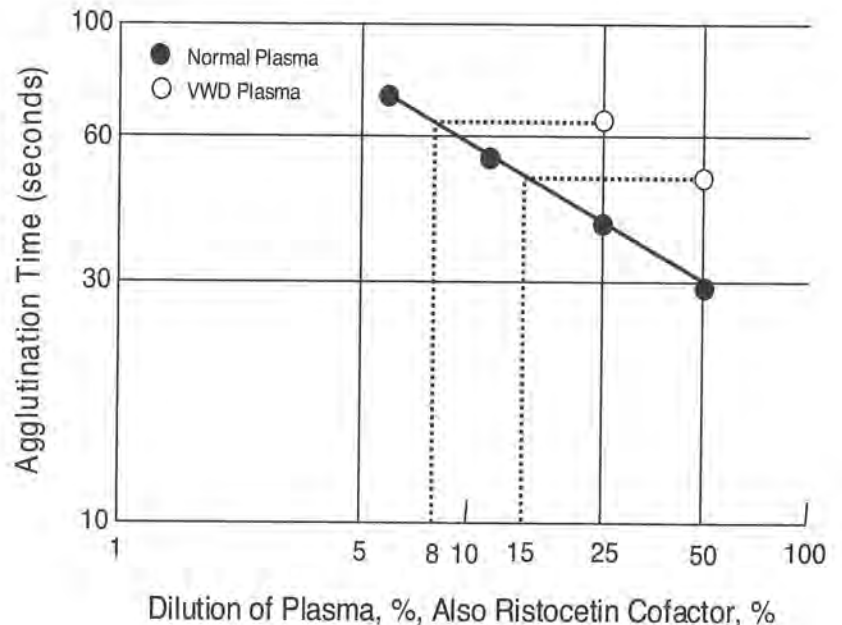


Figure 6. Reference graph for ristocetin cofactor.

*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%;  $15 \times 2 = 30\%$  VWF:RCo.*

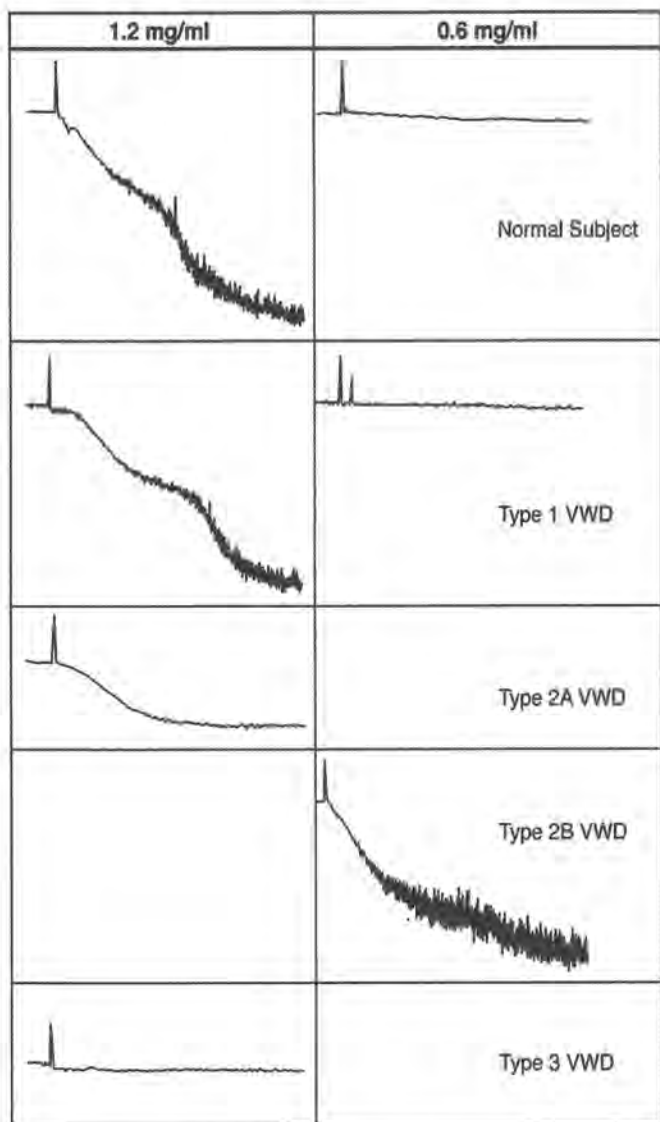
*His plasma diluted to 25% (1:4) has an agglutination time similar to normal plasma diluted to 8%;  $8 \times 4 = 32\%$  VWF:RCo.*



concentrations. The higher of the two ristocetin concentrations is one that always stimulates aggregation of normal platelet-rich plasma, the lower concentration is one that never stimulates aggregation of normal platelet-rich plasma. Use of the second, lower concentration of ristocetin reveals any tendency to over-respond (as in type 2B) 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 it is robust at the lower concentration of ristocetin in type 2B VWD and in pseudo-VWD (Figure 7).

ELISA tests measuring **VWF binding to collagen**, VWF:CB, reflect another VWF function. The test is popular in Australia, used in Europe, but not widely used in the USA. VWF:CB is low in all the 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:FVIII, is available in a few laboratories. In type 2N VWD, binding is virtually absent. Heterozygotes



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. High-resolution gels display abnormal band patterns in unusual types of VWD. Most multimer analysis is performed at reference laboratories.

### Choice of diagnostic tests

In the USA, initial diagnostic tests usually include assays of FVIII, VWF:RCo and VWF:Ag. In Australia and in a few other places, VWF:RCo may be substituted by, or supplemented by, VWF:CB. The BT, PFA-100® or RIPA are performed if locally available. Additional tests, such as multimer analysis and VWF:FVIII B, are usually performed at specialized reference laboratories if needed.

The most useful and critical test, VWF:RCo, has become difficult to obtain, primarily because it was difficult to adapt to automation. The original versions, primarily manual, could be done in local coagulation laboratories, thus, were easily available and could be repeated frequently, e.g., during the post-operative period. Nowadays, the test usually is sent to a reference laboratory.

VWD is difficult to diagnose accurately. My preference would be to obtain all the above tests when evaluating a new patient, together with tests for other bleeding disorders, such as platelet disorders.

## 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** advisable 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 Gelfoam® (absorbable gelatin sponge), sometimes fortified with thrombin powder. Fibrin glue has been used in tooth sockets.

**Figure 7. 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, who has baseline FVIII and VWF:RCo levels of 10%, also is the subject of figure 7. 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.

have intermediate levels. Persons with type 1 or other type 2 VWD have normal levels.

ELISA tests for **von Willebrand factor antigen**, VWF:Ag, using antibodies to VWF, are commonplace. The test measures the total amount of the protein, not its function, thus, in type 2 VWD, levels of VWF:Ag typically are higher than are levels of VWF:RCo or VWF:CB.

VWF is electrophoresed in gels to demonstrate differential migration of **multimers** of different sizes. Antibodies to VWF mark the multimers, along with a label that creates a visible record. Gels with

### Avoid anti-platelet agents

In normal persons, ingestion of aspirin doubles the Ivy bleeding time. Aspirin may greatly prolonged the BT and accentuate clinical bleeding in persons with 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

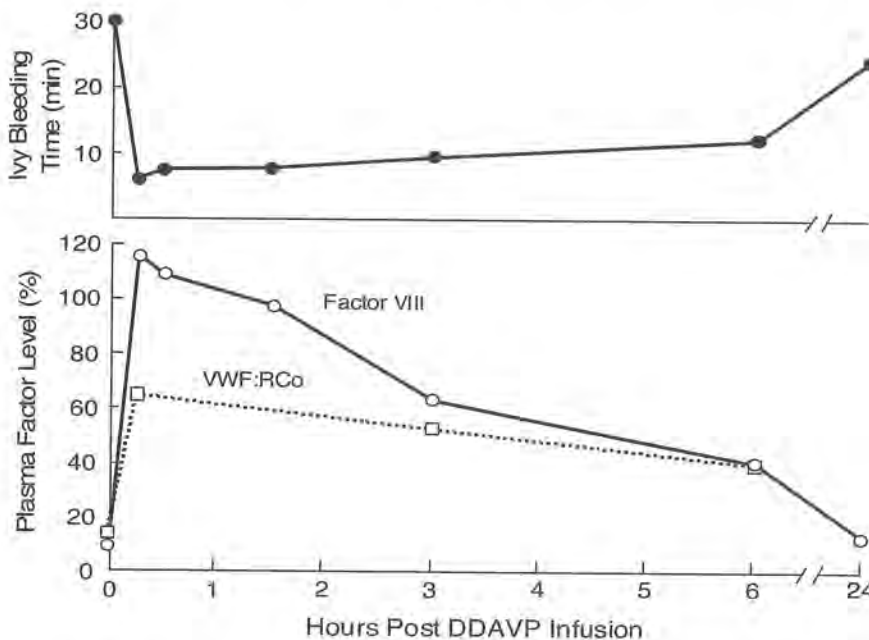
Epsilon-amino-caproic-acid (EACA, Amicar®) and tranexamic acid (Cyclokapron®) both inhibit plasminogen activator, thus preventing formation of plasmin, which lyses fibrin. They are often highly-effective for control of mouth or nose bleeding, for menorrhagia and for dental extractions. They sometimes are adequate as sole therapy, or, they may be combined with DDAVP or clotting factor concentrate.

Ten grams/day of EACA, in divided doses, are given orally to adults. Three to four grams of tranexamic acid, in divided doses, are given orally to adults. Higher doses have been used for menorrhagia (see bibliography). Intravenous formulations also are available, e.g. for use before surgical procedures.

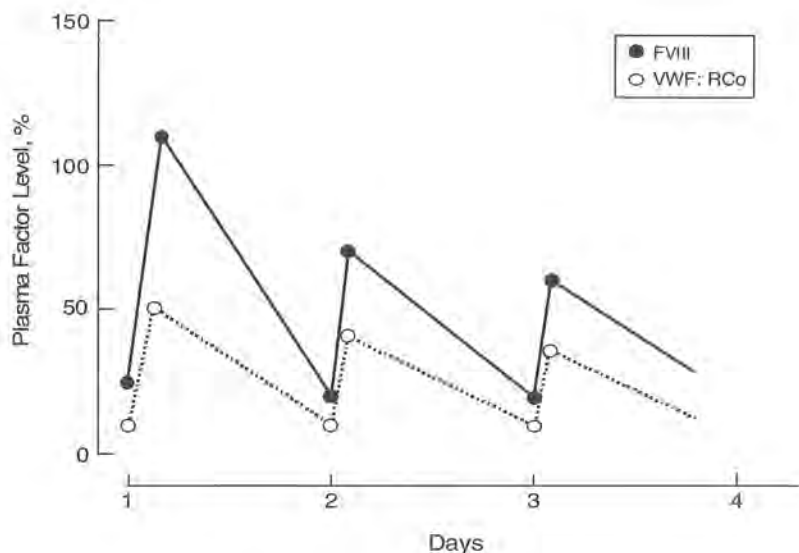
### DDAVP

DDAVP (deamino-8-D-arginine vasopressin, desmopressin, Stimate®) 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 subcutaneous route also can be used.) Some persons with types 1 and 2 VWD have similar responses (Figure 8), but some have lesser responses (related, in part, to genotype). The level of response on different occasions tends to be similar within a given person and kindred. A test dose often is given after VWD is diagnosed to define a given patient's responsiveness. If doses are closely-spaced, however, e.g. every 24 hours, the response to the second and later doses may be less than the response to the first dose (tachyphylaxis), as would be expected if stores of FVIII and VWF must be replen-



**Figure 8. 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 9. Tachyphylaxis. Hypothetical 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.**

ished (Figure 9). Dilute DDAVP nosedrops used for diabetes insipidus are inadequate in VWD.

DDAVP is used to stop acute bleeding in responsive patients with type 1 VWD and in some with type 2A or 2M VWD. The bleeding time may be corrected temporarily in type 1 VWD and improved in type 2A or 2M. DDAVP suffices to prevent excessive surgical bleeding in most patients with type 1 VWD who are responsive to the drug. The standard dose may be repeated daily.

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 of the efficacy of DDAVP stems from its direct effect on platelets. It may be useful for mucosal bleeding and tooth extractions even if the levels of FVIII and VWF:RCo do not rise.

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 anti-fibrinolytic 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 infarction have been reported, especially in older patients with many risk factors for such events. Many patients who did not have bleeding disorders were given DDAVP to reduce blood loss in major surgery. Two meta-analyses of large controlled clinical trials in such patients yielded opposite opinions on the contribution of DDAVP to these events. Many clinicians remain uneasy about giving DDAVP to their older patients, with and without VWD.

### Plasma and cryoprecipitate

Fresh-frozen or lyophilized plasma, or cryoprecipitate, were used in the past to treat patients with VWD who did not have adequate responses to DDAVP. Because of the residual risk of transmitting viral infections with single-donor plasma products, viral-inactivated concentrates are now preferred. Occasionally, the needs of an individual patient are still met with cryoprecipitate or plasma from one or more well-screened donors who are plasmapheresed repeatedly. Some donors receive DDAVP before donation to raise their levels of FVIII and VWF.

Plasma or cryoprecipitate immediately elevates the patient's levels of FVIII and VWF and may or may not improve the Ivy BT. In some patients, the BT cannot be corrected with large doses of cryoprecipitate. Nevertheless, hemostasis is excellent, even in surgical procedures. The half-life of the infused VWF:RCo is about 10 hours. In contrast, the level of FVIII continues to rise because the exogenous VWF is available to transport the patient's endogenously-produced FVIII.



If plasma is promptly separated from red cells and frozen, it retains HMW multimers and contains about one unit of FVIII and of VWF:RCo per milliliter. The volume of plasma that can be infused at any one time is limited by the capacity and elasticity of the vasculature. A reasonable volume for a middle-aged 70 kg patient would be 500 ml of plasma containing 500 units of FVIII and 500 units of VWF:RCo, or, 7 units/kg. That volume would raise his FVIII and VWF:RCo level by 14 percentage-points (e.g. from zero to 14%.) A larger volume of plasma, perhaps 750 ml, might be given to a young slender patient with a healthy heart and lungs. When it was commonly used, plasma was given pre-operatively, again after the surgical procedure, and, in the post-operative period, once or twice-daily for severe VWD and once-daily for moderate VWD. Its efficacy was generally very good.

Cryoprecipitate also retains HMW multimers. In the USA, cryoprecipitate made from plasma recovered from individual whole blood donations contains, on average, 80 units of FVIII and of VWF:RCo per bag. The volume in each bag is trivial so volume-overload is not a concern. Many bags can be given to provide a high dose of FVIII and VWF, if desired. The dosage schedule I used for patients with type 3 VWD for major surgery was 50 FVIII U/kg pre-operatively (achieving a 100% plasma FVIII level) and half that amount, given every 12 hours, afterwards. Patients with milder VWD were given cryoprecipitate at 24 hour intervals after surgery. The dosages used provided excellent hemostasis and probably were overly generous.

### VWF-FVIII concentrates

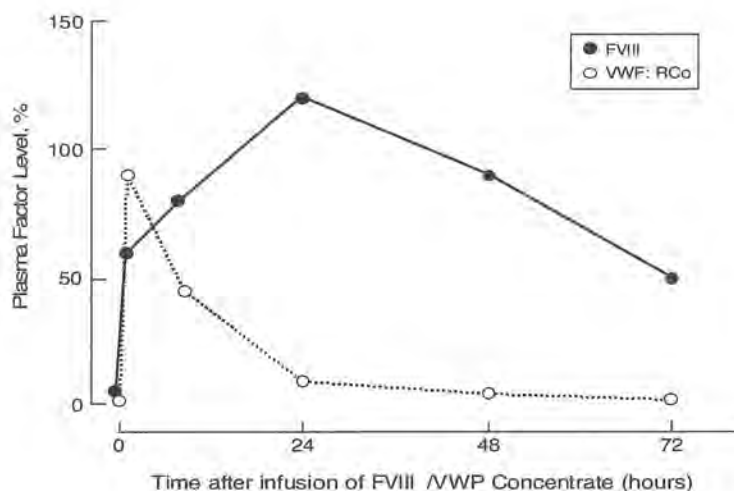
A variety of plasma-derived concentrates of

FVIII with VWF have been used to treat VWD patients unresponsive to DDAVP (Figure 10). The concentrate most widely used, Humate-P® (also spelled Haemate-P and Hemate-P), made in Germany by CSL Behring, has more than two international units (IU) of VWF:RCo per IU of FVIII and retains HMW multimers well. The CSL product Biostate®, made in Australia, has similar characteristics. The new French product Wilstart® combines a given number of units of FVIII from their FVIII concentrate Factane® with twice as many VWF:RCo units from their VWF concentrate Wilfactin® (see page 16). Alphanate®, made in the USA by Grifols, contains about 0.5-1.0 IU of VWF:RCo per IU of FVIII; HMW multimers are not well-retained. Koate DVI®, made in the USA by Talecris, and Immunate®, made in Vienna by Baxter, also contain less VWF:RCo than FVIII and lack HMW multimers. Other FVIII-VWF concentrates have been used in other countries.

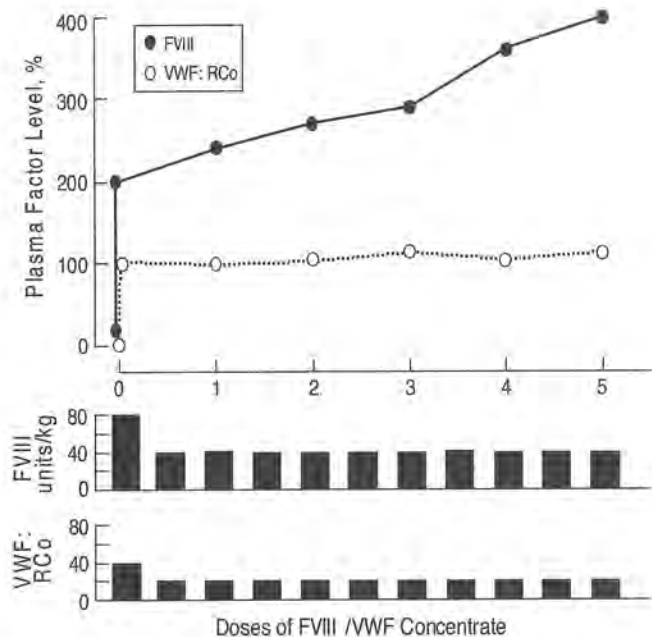
Early publications on the use of FVIII-VWF products reported dosage in terms of FVIII international units (IU) whereas recent reports quote dosage in VWF:RCo IU. (International standards for VWF measurements were developed recently.)

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.

All the products named above were reported to be highly effective in controlling bleeding episodes and preventing excessive bleeding in surgery. The generous dosages described may have obscured possible differences in efficacy among these products. In recent clinical trials of FVIII-VWF concentrates for surgery, the chosen dosages kept the pa-



**Figure 10. Response of FVIII and VWF:RCo in a patient with type 3 VWD after a single infusion of FVIII-VWF concentrate.**



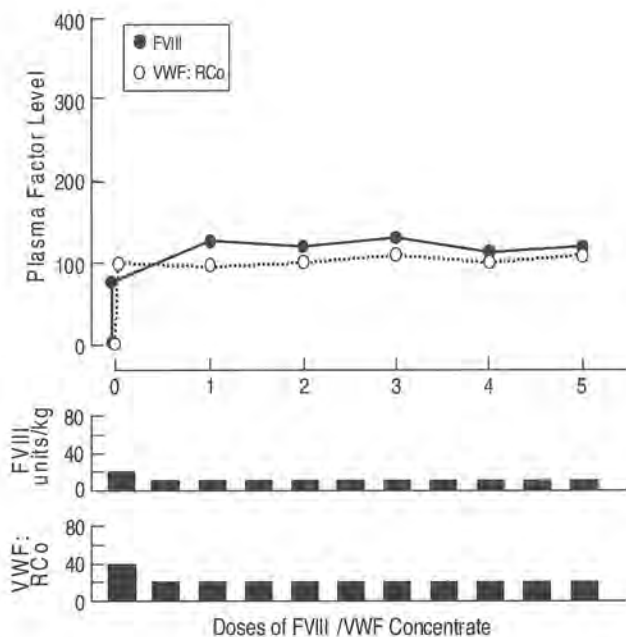
**Figure 11. Surgical dosage, using a concentrate with more FVIII than VWF. Hypothetical mean daily factor levels in a patient with severe VWD treated for surgery with an initial dose (40 IU of VWF:RCo/kg and 80 IU of FVIII/kg) and subsequently with doses half that much every 12 hours, with the goal 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 limits.**

patient's plasma level of VWF:RCo close to 100%. Total levels of VWF:Ag, reflecting both large and small multimers, were much higher than levels of VWF:RCo. Small multimers can bind and carry FVIII. Patients' plasma levels of FVIII rose higher than did levels of VWF:RCo. The total FVIII level consists of infused exogenous FVIII plus endogenously-released FVIII. Plasma levels of FVIII sometimes greatly exceeded the upper limit of normal when concentrates with more FVIII than VWF were given.

Figures 11 and 12 were constructed from 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%.

Patients with type 1 VWD who are inade-



**Figure 12. Surgical dosage, using a 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.**

quately responsive 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 2A, B, M or N VWD, VWF is qualitatively abnormal. I consider the level of VWF:RCo to be zero for the sake of calculating dosage in type 2. To raise the level of VWF:RCo from a baseline of zero (as in type 3 VWD), or effectively near zero (as in type 2), to a desired level of 50%, the patient should receive about 25 IU VWF:RCo/kg.

A single dose of concentrate, sufficient to raise the plasma level of VWF:RCo to 50%, is very generous treatment for most acute hemorrhages, deep injections, and simple dental extractions. Lower doses may suffice. For surgical operations, an initial dose may be given that raises the level of VWF:RCo to 100% and subsequent doses, half the loading dose, may be given every 12–24 hours to maintain the VWF:RCo level above 50% in severe VWD, with lesser doses or less frequent doses in mild VWD. The half-life of infused VWF:RCo is faster in type 3 than in type 2, and

faster in type 2 than in type 1, according to a recent report. It is preferable to follow levels of VWF:RCo post-operatively, but that option is available in a decreasing number of hospitals, unfortunately. Patients with bleeding or surgical operations in the nose, mouth or throat, and patients having dental extractions, who are receiving concentrates the post-operative levels with VWF:RCo levels, but that option is decreasingly available. The duration of treatment depends on the type of surgery performed. Treatment for a day or two suffices for minor procedures. I treat patients having major procedures operations for 10 days, the same as patients with hemophilia.

Some clinicians advise also following the FVIII level to make sure it does not rise above the normal range. A slightly-increased incidence of post-operative deep vein thrombosis in VWD, compared to hemophilia A, has been blamed on the very high FVIII levels sometimes seen. Some clinicians measure only FVIII levels, a test more widely available than VWFRCo, and are satisfied if the FVIII level is about 100% during the operation and between 50 and 100% thereafter (as in hemophilia A.)

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

Concentrates also may be given **prophylactically** during menses in women with severe menorrhagia or for longer terms in patients with other serious recurrent bleeding problems, such as severe epistaxis, or joint hemorrhages, or gastrointestinal bleeding. Concentrates may be given at the time of delivery of women with type 2 or 3 VWD or moderately severe type 1.

Concentrates containing FVIII alone, such as those made from plasma using monoclonal-antibody affinity chromatography, and all recombinant FVIII concentrates, may help control bleeding in patients with VWD when no concentrate containing VWF is available. Such concentrates are considered sub-optimal therapy for VWD. The rare patients with inhibitors to VWF may be given concentrates of FVIII alone and derive some benefit from them.

### Concentrates of VWF

LFB in France has produced a plasma-derived VWF concentrate, Wilfactin®, with very low levels of FVIII and with excellent preservation of HMW multimers. 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 to express and circulate his own endogenous FVIII) and a second dose immediately pre-operatively. For urgent surgery, the initial dose of VWF concentrate is accompanied by a dose of a FVIII concentrate. Alternatively, the new product, Wilstart®, which combines Wilfactin® and the FVIII concentrate Factane®, is used.

Elsewhere, a recombinant VWF concentrate was developed and used successfully in dogs. The product has not yet entered human clinical trials..

### Platelets

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

### Menorrhagia

Control of menorrhagia may require multiple agents. Estrogen-progesterone pills and anti-fibrinolytic agents may be tried first. Antifibrinolytic drugs may be effective in larger doses than those given for oral surgery (see references.) If these agents are insufficient, DDAVP nasal spray may be tried. If all these agents are insufficient, then concentrates may be needed. The care of a sympathetic and persistent gynecologist is invaluable. 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 or local agents. Anti-fibrinolytic drugs are the main line of defense against mucosal bleeding and are given prophylactically. For invasive dental procedures, DDAVP or concentrate is usually given in addition to anti-fibrinolytic agents. Tooth sockets may be packed with local hemostatic agents.

Be wary of injections for **regional block anesthesia**. Accidental piercing of a small vessel in the neuro-vascular bundle behind the angle of the jaw can provoke a hematoma that can dissect down the neck rapidly and compress the trachea. Preparation with DDAVP or concentrate is advisable, as for a minor surgical procedure.

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## Terminology

Ruggeri ZM, Mannucci PM, Lombardi R, Federici AB, Zimmerman TS. Multimeric composition of factor VIII/von Willebrand factor following administration of DDAVP: Implications for pathophysiology and therapy of von Willebrand's disease subtypes. *Blood* 1982; 59: 1272-1278.

The terminology "Types I, IIA, IIB and III" is 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, with outstanding figures.)

Mazurier C, Rodeghiero F. Recommended abbreviations for von Willebrand factor and its activities. *Thromb Haemost* 2001; 86:712.

The Subcommittee on VWF, Scientific and Standardization Committee, International Society on Thrombosis and Hemostasis recommended abbreviations for VWF and its activities as follows:

ATTRIBUTE	RECOMMENDED ABBREVIATION
Mature protein	VWF
Antigen	VWF:Ag
Ristocetin cofactor activity	VWF:RCo
Collagen binding activity	VWF:CB
Factor VIII binding capacity	VWF:FVIIIb

Sadler JE. A Revised Classification of von Willebrand disease: For the Subcommittee on von Willebrand factor of the Scientific and Standardization Committee of the International Society on Thrombosis and Hemostasis. *Thromb Haemost* 1994; 71:520-525.

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 function was clearly abnormal. The new classification declared that all VWD is caused by mutations at the VWF gene locus. 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 types that had been described in the previous decade were gathered into major types. Arabic numerals were used instead of the former Roman numerals, to distinguish types. Types 1 and 3 were described as quantitative and type 2 as qualitative, as follows:

Type 1	Partial quantitative deficiency of VWF (large multimers present in plasma, with or without low levels of VWF in platelets. The structure of VWF subunits is normal.
Type 2 A	Decreased platelet-dependent function due to absence of large multimers of VWF
Type 2 B	Increased affinity of VWF for platelet glycoprotein Ib. (Increased RIPA at low concentrations is characteristic but not present in every case.) Large multimers of VWF may not be absent from plasma in all cases.
Type 2 M	VWF has decreased platelet-dependent function but large multimers are present, sometimes larger-than-normal multimers are present. ("M" is for "multimer".) This type includes the "Vicenza" (Italy) variant with larger-than-normal multimers.
Type 2 N	VWF has markedly-decreased affinity for factor VIII. ("N" is for the "Normandy" patient discovered in France.
Type 3	There is a virtually complete deficiency of VWF.

Sadler JE, Matshushita T, Dong Z, Tuley EA, Westfield FA. Molecular mechanism and classification of von Willebrand disease. *Thromb Haemost* 1995; 74:161-164. *Details of 1994 classification, supposed pathogenesis.*

Goodeve AC, Eikenboom JCJ, Ginsburg D, Hilbert L, Mazurier C, Peake IR, Sadler JE, Rodeghiero F on behalf of the ISTH SSC subcommittee on von Willebrand factor. A standard nomenclature for von Willebrand factor gene mutations and polymorphisms. *Thromb Haemost* 2001; 85:929-931.

The VWF cDNA 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. To avoid confusion with previously used numbering schemes for mature VWF, which started from serine 764 of pre-pro VWF, the use of the single letter amino acid code is recommended.

Sadler JE and many others. Update on the pathophysiology and classification of von Willebrand disease: a report of the Subcommittee on von Willebrand Factor. *J Thromb Haemost* 2006; 4:2103-2114.

The 1994 classification of VWD into types 1, 2A, 2B, 2M, 2N and 3 was retained. Types 1 and 2A are caused by various pathophysiological mechanisms. Further studies may justify subdivision of these types.

## History, early descriptions

Von Willebrand, EA. Hereditary pseudohaemophilia. *Haemophilia* 1999; 5:223-231.

This is an English translation of the original article, which appeared in Swedish in 1926. Dr. von Willebrand referred to 19 cases in the prior literature of a hemorrhagic disorder differing from hemophilia, appearing more often in women than in men. His own patient was a girl from one of the Åland islands (between Sweden and Finland) brought to Helsinki for his consultation. The most frequent type of bleeding among 22 affected

family members was epistaxis, followed by profuse bleeding from oral lesions, easy bruising and, in females, excessive bleeding with menses and at childbirth. Intestinal bleeding had been a cause of death in some family members. The girl had a normal platelet count but a very prolonged Duke bleeding time. The BT also was prolonged in other severely-affected family members but not in all mildly-affected ones. A capillary-fragility test was positive (that is, petechiae appeared on the forearm when a blood-pressure cuff on the upper arm was inflated to a level between systolic and diastolic pressure for several minutes.) Whole blood clotting times were normal. The inheritance appeared to be dominant with instances of incomplete penetrance. The index patient and those of her siblings who also were severely affected were probably homozygotes, being products of a consanguineous union. Dr. von Willebrand believed that the disorder was due to "a disturbed function of the platelets and a general lesion of the capillary walls."

Minot G. A familial hemorrhagic condition associated with prolongation of the bleeding time. *Am J Med Sci* 1928; 175:301-306.

Symptomatic persons in two families had prolonged bleeding times and such symptoms as epistaxis, bruising and prolonged bleeding from small cuts. Male to male transmission was noted in one family, ruling out sex-linked inheritance. The platelet count and the whole blood clotting time were normal. Dr. Minot's name was associated with the condition in the USA in early years.

### History, development of comprehension of nature of VWD

Many excellent papers in the 1950's and 1960's from continental Europe were published in the languages of the authors but only English-language papers are cited below. In early years, VWD was recognized only in patients with long bleeding times, probably those who now would be described as having fairly severe type 1, or type 2A or B, or type 3 VWD. We now know the pattern of relationships of FVIII and VWF:Ag and VWF:RCO levels in these types, but the variation was confusing to early investigators.

### Reviews

Mammen EF. Von Willebrand's disease: history, diagnosis and treatment. *Sem Thromb Hemost* 1975; 2:61-84. Review, 180 references.

By the time of this outstanding review, evidence had developed that the "factor VIII molecule" was composed of subunits which aggregated, and that two molecular species were involved, a small one with "biological activity" and a larger "carrier protein".

Ruggeri ZM, Zimmerman TS. Von Willebrand factor and von Willebrand disease. *Blood* 1987; 70:895-904.

This review contains a useful table of additional "subtypes" and their defining attributes, gathered since the official nomenclature of 1984. The 1994 nomenclature lumped several of these types together. Subsequent discovery of corresponding mutations makes these unusual variants interesting again.

Nilsson IM. The history of von Willebrand disease. *Haemophilia* 1999. 5 (suppl 2): 7-11. *Studies of the disorder in the Åland islands and Sweden.*

### Cross-transfusion experiments

These experiments were performed in the 1950's and 60's before the potential for spreading infection through transfusion was understood well. Those described below were representative, not the sole instances.

Nilsson IM, Blomback M, Jorpes E, Blomback B, Johansson SA. V.Willebrand's disease and its correction with human plasma fraction I-O. *Acta Med Scand* 1957; 159:179-188

In this first report in English, the authors describe infusion of Cohn's plasma fraction I-O (which contained FVIII and VWF) into patients with VWD from the Åland islands. The infusions elevated FVIII levels and correction prolonged Duke BTs.

Nilsson IM, Blomback M, Blomback B. v.Willebrand's disease in Sweden. Its pathogenesis and treatment. *Acta Med Scand* 1959; 164: 263-278.

In Sweden, a girl with severe VWD, baseline FVIII level 6%, was treated for hemorrhages with Cohn fraction I-O prepared from normal plasma. On one occasion she was transfused with fraction I-O made from the pooled plasma of patients with severe hemophilia A. Two hours after the latter infusion, 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-O made from the plasma of patients with VWD and her FVIII level did not rise.

Cornu P, Larrieu MJ, Caen J, Bernard J. Transfusion studies in von Willebrand's disease: Effect on bleeding time and factor VIII. *Brit. J. Haemat* 1963; 9:189-202.

In France, patients with hemophilia A served as donors of fresh blood (in six instances) or plasma (in two instances) 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. The authors noted that there is no cross-correction of plasmas from VWD and hemophilia A patients *in vitro* and only unilateral correction *in vivo*.

Lewis JH. Synthesis of AHF in von Willebrand's disease. *Blood* 1964; 23:233-238.

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

Howard MA, Firkin BG. Ristocetin - A new tool in the investigation of platelet aggregation. *Thromb Diath Haemorrh* 1971; 26: 362-369.

Ristocetin was an antibiotic 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 (moderate to severe) VWD. They recognized the potential utility of ristocetin aggregation in diagnosing VWD. (*They did a ristocetin-induced platelet aggregation test, RIPA, which is usually deficient in type 2A and type 3 VWD.*)

Howard MA, Sawers RJ, Firkin BG. Ristocetin: A means of differentiating von Willebrand's disease into two groups. *Blood* 1973; 41:687-690.

Fourteen patients with VWD from nine families were studied. All had prolonged BTs (at least on some occasion), and FVIII levels below normal. The nine patients who had absent or very low adhesiveness of platelets to glass also had absent or very low RIPA (and probably had type 2A VWD). Those five patients with borderline adhesiveness had normal or near-normal RIPA (and probably had type 1 VWD). Related patients behaved similarly in these tests, suggesting genetic groups. 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 normally present in plasma (rather than a factor in platelets) was deficient in VWD.

Meyer D, Jenkins CSP, Dreyfus M, Larrieu MJ. Experimental model for von Willebrand's disease. *Nature* 1973; 243: 293-294.

RIPA was absent or definitely reduced in eight patients with VWD. A semi-purified FVIII concentrate, made from normal plasma, corrected the defective platelet adhesiveness to glass seen in patients with 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 (i.e. to VWF). The antibody inhibited the ristocetin-induced aggregation of normal platelet-rich plasma. The authors concluded that plasma von Willebrand factor was needed to support ristocetin aggregation.

Weiss HJ, Rogers J, Brand H. Defective ristocetin-induced platelet aggregation in von Willebrand's disease and its correction by factor VIII. *J Clin Invest* 1973, 52:2697-2707.

RIPA 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.

The authors 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.

Weiss HJ, Hoyer LW, Rickles FR, Varma A, Rogers J. Quantitative assay of a plasma factor deficient in von Willebrand's disease that is necessary for platelet aggregation: Relationship to factor VIII procoagulant activity and antigen content. *J Clin Invest* 1973; 52: 2708-2716.

An assay for VWF activity was described, in which washed normal platelets were combined with various dilutions of normal plasma and aggregation was stimulated by ristocetin (the ristocetin co-factor test). A linear relationship (on log-log transformation) existed between the amount of ristocetin-induced aggregation and the degree of dilution of the normal plasma. 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. Low levels of ristocetin cofactor correlated better with prolonged BTs than did levels of VWF:Ag (which had just been described) or of FVIII, suggesting that ristocetin co-factor might be the anti-bleeding factor deficient in VWD.

Read MS, Shermer RW, Brinkhous KM. Venom coagglutinin: An activator of platelet aggregation dependent on von Willebrand factor. *Proc Natl Acad Sci USA* 1978; 75:4514-4518.

A platelet aggregating activity was found in many snake venoms, predominantly those of the genus *Bothrops*. The activity was found only in the presence of von Willebrand factor. A snake-venom reagent called "Botrocetin" was developed to test for VWF activity. (There was hope that this reagent would substitute for ristocetin, which, after withdrawal from clinical use, became unobtainable for a while.)

#### Discoveries using immunologic tests

Feinstein D, Chong MNY, Kasper CK, Rapaport SI. Hemophilia A: Polymorphism detectable by a factor VIII antibody. *Science* 1969; 163:1071-1072.

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 these results with those described next.)

Stites DP, Hershgold EJ, Perlman JD, Fudenberg HH. Factor VIII detection by hemagglutination inhibition: Hemophilia A and von Willebrand's disease. *Science* 1971; 171:196-107.

"Factor VIII" (believed, at the time, to be highly-purified) was injected into rabbits who made antibodies to it. (In reality, the rabbits made antibody to VWF.) Using this antibody, "factor VIII" (in reality, VWF) was detected in the plasma of 14 normal persons and 14 persons with hemophilia A. The authors concluded that persons with hemophilia A made a FVIII molecule that lacked function. In contrast, "factor VIII" could not be detected in the plasma of six patients with VWD and was detected only at a low level in two other patients with VWD.

Zimmerman TS, Ratnoff OD, Powell AE. Immunologic differentiation of classic hemophilia (factor VIII deficiency) and von Willebrand's disease. *J Clin Invest* 1971; 50:244-254.

"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.

Hoyer LW. Immunologic studies of antihemophilic factor (AHF, factor VIII.) III. Comparative binding properties of human and rabbit anti-AHF. *Blood* 1972; 39: 481-489.

Bound, radio-labelled rabbit antibody raised against so-called "purified factor VIII" was used to assay an antigen found in normal and in hemophilia A plasma but markedly reduced in VWD. An auto-antibody against FVIII (which had arisen in a non-hemophilic patient) did not react

with the antigen recognized by the rabbit antibody.

Bennett B, Ratnoff OD, Levin J. Immunologic studies in von Willebrand's disease: Evidence that the antihemophilic factor (AHF) produced after transfusions lacks an antigen associated with normal AHF and the inactive material produced by patients with classic hemophilia. *J Clin Invest* 1972; 51:2597-601.

Two patients with VWD were transfused with normal plasma. FVIII activity rose immediately and, in one instance in which assays were followed, stayed elevated for three days. In contrast, levels of the "AHF-like antigen" detected by rabbit antibodies peaked early and fell to, or near, baseline by 24 hours. The authors proposed that the plasma protein deficient in VWD stimulates production of FVIII.

Holmberg L, Nilsson IM. Genetic variants of von Willebrand's disease. *Br Med J* 1972; 822:317-320.

VWF:Ag was measured in 70 VWD patients from 23 families in an early (unsuccessful) attempt to categorize patients according to its level. The authors comment that the entities probably consists of "AHF residing in or complexed with the von Willebrand factor".

Jaffe EA, Hoyer LW, Nachman RL. Synthesis of antihemophilic factor antigen by cultured human endothelial cells. *J Clin Invest* 1973; 52:2757-2764

"Factor VIII antigen" (later termed VWF:Ag), identified by radio-labeled antibodies of rabbit origin, was located within cultured endothelial cells and in the culture medium around these cells. By tracing radio-labeled amino acids incorporated into the "factor VIII antigen", the authors showed that endothelial cells synthesize and release a protein that shares antigen present on normal human "factor VIII". No FVIII coagulant activity, however, was found in the culture medium. One hypothesis was that endothelial cells synthesize only one component of a molecular complex that also has FVIII coagulant activity. Production of the antigen in endothelial cells, lining vascular channels, was viewed as possibly important to its role in hemostasis.

Hoyer LW, De los Santos RP, Hoyer JR. Antihemophilic factor antigen. Localization in endothelial cells by immunofluorescent microscopy. *J Clin Invest* 1973; 52:2737-1744

Using immunofluorescent labeling and rabbit antibodies, VWF:Ag was visualized in platelets, in megakaryocytes and in endothelial cells of many organs.

Howard MA, Montgomery DC, Hardisty RM. Factor-VIII-related antigen in platelets. *Thromb Res* 1974; 4:617-624

Normal human platelets were shown to contain VWF:Ag which did not exchange with plasma VWF:Ag. Platelets from patients with VWD or with thrombasthenia were deficient in VWF:Ag whereas those from patients with the Bernard-Soulier syndrome had greater than normal amounts of VWF:Ag.

Sultan Y, Bouma BN, de Graaf S, Simeon J, Caen JP, Sixma JJ. Factor VIII related antigen in platelets of patients with von Willebrand's disease. *Thromb Res* 1977; 11:23-30.

VWF:Ag could not be detected in the platelets of seven patients with severe VWD who also had undetectable VWF:Ag in their plasma. VWF:Ag could be detected in normal amounts in the platelets of 9 of 10 patients who did have measurable VWF:Ag in the plasma.

Slot JW, Bouma BN, Montgomery R, Zimmerman TS. Platelet factor VIII-related antigen: Immunofluorescent localization. *Thromb Res* 1978; 13:871-881.

The antigen (VWF:Ag) was found in granules in the platelet and not detected on the surface or membrane.

Piovella F, Nalli G, Malamani GD, Majolino I, Frassoni F, Sitar GM, Ruggeri A, Dell'Orbo C, Ascarì E. The ultrastructural localization of factor VIII-antigen in human platelets, megakaryocytes and endothelial cells, utilizing a ferritin-labelled antibody. *Brit J Haematol* 1978; 39:209-213.

"Factor VIII antigen" (VWF:Ag) was found in endothelial cells and megakaryocytes, and was presumed to be synthesized there. Its presence in platelets is probably derived from megakaryocytes.

Rand JH, Sussman II, Gordon RE, Chu SV, Solomon V. Localization of factor-VIII-related antigen in human vascular subendothelium. *Blood* 1980; 55:752-6.

**VWF:Ag in human vascular subendothelium was visualized with electron microscopy and immuno-fluorescent staining.**

Montgomery RR, Zimmerman TS. Von Willebrand's disease antigen II. *J Clin Invest* 1978; 61: 1498-1507.

**A second VWF antigen was detected by antibodies raised in rabbits. (See next citation.)**

Fay PJ, Kawai Y, Wagner DD, Ginsburg D, Bonthron D, Ohlsson-Wilhelm BM, Chavin SI, Abraham GN, Handin RI, Orkin SH. Propolypeptide of von Willebrand factor circulates in blood and is identical to von Willebrand antigen II. *Science* 1986; 232:995-998.

**Von Willebrand antigen II was recognized as the 100-kilodalton pro-peptide "that is first cleaved from pro-VWF during intracellular processing and then released into plasma."**

### Evidence that FVIII and VWF were two entities

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

**Several hypotheses about the relationship between FVIII and VWF were entertained. VWF might be an activator or a regulator of FVIII production, or, FVIII and VWF might be produced separately and combine. (The latter approximates current understanding. Hypotheses abound, but this is the first publication I have located that contained a correct guess.)**

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

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

Zimmerman TS, Edgington TS: Factor VIII coagulant activity and factor VIII-like antigen: independent molecular entities. *J Exp Med* 1973; 138:1015-1020

**Multiple antibodies against "factor VIII" preparations were raised in rabbits, some affecting VWF:Ag and some affecting FVIII coagulant activity. These antibodies and a human inhibitor against FVIII were fixed to agarose beads. FVIII or VWF:Ag each was adsorbed by its specific antibody, leaving the other entity in the supernatant, thus showing that FVIII and VWF:Ag were separable.**

Weiss HJ, Hoyer LW. Von Willebrand factor: Dissociation from antihemophilic factor procoagulant activity. *Science* 1973; 182:1149-1151.

**"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."**

Counts RB, Paskell SL, Elgee SK. Disulfide bonds and the quaternary structure of factor VIII/von Willebrand factor. *J Clin Invest* 1978; 62:702-709.

**A low-molecular weight (MW about 240,000) coagulant FVIII could be separated from a high molecular weight protein having both FVIII and VWF activities. The two entities were linked by disulfide bonds.**

Tuddenham EG, Trabold NC, Collins JA, Hoyer LW. The properties of factor VIII coagulant activity prepared by immunoabsorbent chromatography. *J Lab Clin Med* 1979; 93: 40-53.

**"Factor VIII" was separated from plasma using an immuno-adsorbent chromatography column. Serine esterase stabilizers were added to preserve FVIII activity. Two entities were obtained, one was FVIII in high concentration and the other the recently-described "factor VIII related antigen" (VWF:Ag).**

### Crossed-immunoelectrophoresis

Peake IR, Bloom AL, Giddings JC. Inherited variants of factor-VIII-related protein in von Willebrand's disease. *N Engl J Med* 1974; 291: 113-117.

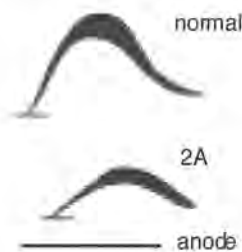
**In Wales, three patients from each of two families had very prolonged BTs and absent RIPA. Their VWF:Ag, present at normal or only mildly-reduced levels, migrated unusually rapidly to the anode on crossed immuno-electrophoresis (that is, electrophoresis was carried out in one direction and then in the perpendicular direction.) These patients differed from typical VWD patients in whom the migration pattern on crossed immuno-electrophoresis was normal. (If all multimers are small, they travel more rapidly than a mixture of small and large multimers. The variant patients had type 2 VWD, lacking large multimers.)**

Kernoff PBA, Gruson R, Rizza CR. A variant of factor VIII related antigen. *Br J Haematol* 1974; 26:435-440.

**In England, the VWF:Ag of a patient with VWD had faster anodal migration on crossed immuno-electrophoresis than did normal plasma or plasma from other patients with VWD.**

Sultan Y, Simeon J, Caen JP: Electrophoretic heterogeneity of normal factor VIII/von Willebrand protein, and abnormal electrophoretic mobility in patients with von Willebrand's disease. *J Lab Clin Med* 1976;87:185-194.

**In France, seven persons with VWD in two families had prolonged BTs, absent RIPA and increased anodal migration of VWF:Ag.**



**Figure 13. Crossed-immunoelectrophoresis. Von Willebrand factor is first electrophoresed in the vertical direction (bottom to top) then in the horizontal direction (left to right towards the anode.)**

### Demonstration of Multimers

Van Mourik JA, Bouma BN, LaBruyere WT, de Graaf S, Mochtar IA. Factor VIII, a series of homologous oligomers and a complex of two proteins. *Thromb Res* 1974; 4:155-164.

**"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, suggesting a series of oligomers the distribution of which depends on the ion strength of the medium. "Factor VIII" could be dissociated into two components with different precipitating properties.**

Fass DN, Knutson GJ, Bowie EJW: Porcine Willebrand factor: A population of multimers. *J Lab Clin Med* 1978; 91:307-320

**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."**

Hoyer LW, Shainoff JR. Factor VIII-related protein circulates in normal human plasma as high molecular weight multimers. *Blood* 1980; 55:1056-1059.

**A series of multimeric forms of VWF:Ag was discovered using SDS agarose electrophoresis. Their size was estimated as MW 0.85 to  $12 \times 10^6$ . The very large forms were found in plasma taken in various anticoagulants, making it likely that the HMW forms existed *in vivo* and were not the result of *in vitro* aggregation, as had been suspected.**

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 platelets. *J Clin Invest* 1980; 65:1318-1325.

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 Xray film to make a visible record. In normal plasma, ten distinct multimer bands were seen, ranging in apparent molecular weight from 0.86 to  $9.9 \times 10^6$ . The dimers and monomers generated by reduction of disulfide bonds were readily identified in this system but were not found in normal plasma or platelets. A normal multimeric pattern (and normal crossed electrophoresis) was seen in patients with type 1 VWD. In plasma from type 2A VWD, only the five smaller multimers were present in significant amounts, sometimes with traces of the 6<sup>th</sup> and 7<sup>th</sup>. Large multimers 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 7<sup>th</sup> and 8<sup>th</sup> multimers easily detected. In type 2B platelets, all normal multimer sizes were represented.

Ruggeri ZM, Zimmerman TS. The complex multimeric composition of factor VIII/von Willebrand factor. *Blood* 1981; 57:1140-1143

"In 1% agarose gels, normal plasma displayed a series of sharply defined oligomers. However, increasing the agarose concentration to 2% or utilizing mixture of 0.8% agarose - 1.75% acrylamide revealed two bands of lesser intensity interposed between the major bands." (Improved methods of looking at multimers would lead to discovery of aberrations in multimer structure in some patients.)

Ruggeri ZM, Mannucci PM, Lombardi R, Federici AB, Zimmerman TS. Multimeric composition of factor VIII/von Willebrand factor following administration of DDAVP: Implications for pathophysiology and therapy of von Willebrand's disease subtypes. *Blood* 1982; 59:1272-1278.

Electrophoresis of VWF:Ag in platelets showed that that large multimers of VWF were present in type 1 and type 2B platelets but not in type 2A platelets. After DDAVP infusion, 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.

Zimmerman TS, Ruggeri ZM. Von Willebrand's disease. *Clin Haematol* 1983; 12:175-200 (The information in the previous paper is presented in more detail, with excellent figures and a general review.)

Hoyer LW, Rizza CR, Tuddenham EGD, Carta CA, Armitage H, Rotblat F. Von Willebrand factor multimer patterns in von Willebrand's disease. *Br J Haematol* 1983; 55:493-507. (contains outstanding figures)

In England, the classification of VWD, according to multimer patterns and RIPA, was described in 116 patients from 47 families

Weiss HJ, Peitu G, Rabinowitz R, Girma JP, Rogers J, Meyer D. Heterogeneous abnormalities in the multimeric structure, antigenic properties and plasma-platelet content of factor VIII/von Willebrand factor in subtypes of classic (type I) and variant (IIA) von Willebrand's disease. *J Lab Clin Med* 1983; 101:411-25

SDS-agarose-acrylamide gel electrophoresis was used to study VWF:Ag. In normal plasma, nine to ten clearly-resolved bands were found, as well as unresolved higher-molecular-weight material. In 11 patients with type 1 VWD, the multimeric structure of VWF:Ag was normal. When levels of VWF:Ag in plasma and in platelets were compared, three subgroups were distinguished: a group with decreased VWF:Ag content in plasma and in platelets, a group with low VWF:Ag levels in plasma but normal levels in platelets, and a group with normal VWF:Ag levels in plasma but decreased levels in platelets.

### Other observations

Tschopp TB, Weiss HJ, Baumgartner HR. Decreased adhesion of platelets to subendothelium in von Willebrand's disease. *J Lab Clin Med* 1974; 83:296-300.

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.

Weiss HJ, Sussman II, Hoyer LW. Stabilization of factor VIII in plasma by the von Willebrand factor. Studies on posttransfusion and dissociated factor VIII and in patients with von Willebrand disease. *J Clin Invest* 1977; 60:390-404.

The lability of FVIII was studied by incubating diluted plasma at 37°C for six hours. When normal plasma was incubated for six hours, an average of 77% of the original FVIII activity remained. A woman with type 3 VWD was transfused with cryoprecipitate. The FVIII in her plasma taken 24-48 hour after transfusion was as labile as that of normal plasma. The FVIII in her plasma taken 72-96 hours after transfusion was more labile, retaining only 35-55% of the original FVIII activity after incubation. Increased lability of FVIII also was seen in three patients with mild VWD. Purified normal VWF or hemophilic plasma added to the VWD plasma samples improved the stability of FVIII. FVIII dissociated from VWF:Ag was more stable when suspended in plasma from a hemophilia A patient than in plasma from a VWD patient. VWF appeared to stabilize FVIII *in vitro* and perhaps also *in vivo*. The authors proposed that FVIII, made under the control of a gene on the X chromosome, was stabilized by VWF, a hypothesis that would explain the post-transfusion findings in VWD.

Gralnick HR, Williams SB, Morisato DK. Effect of the multimeric structure of the factor VIII/von Willebrand protein on binding to platelets. *Blood* 1981; 58:387-97

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

Ruggeri ZM, DeMarco L, Gatti L, Bader R, Montgomery RR. Platelets have more than one binding site for von Willebrand factor. *J Clin Invest* 1983; 72:1-12

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. Platelets have two binding sites for VWF.

Morton LF, Griffin B, Pepper DS, Barnes MJ. The interaction between collagens and factor VIII/von Willebrand factor: investigation of the structural requirements for interaction. *Thromb Res* 1983;32: 545-556.

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

Kessler CM, Floyd CM, Rick ME, Krizek DM, Lee SL, Gralnick HR. Collagen-factor VIII/von Willebrand factor protein interactions. *Blood* 1984; 63:1291-8.

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 acted as a subendothelial collagen-platelet bridge.

Lynch DC, Williams RW, Zimmerman TS, Kirby EP, Livingston DM. Biosynthesis of the subunits of factor VIII by bovine aortic endothelial cells. *Proc Natl Acad Sci* 1983; 80:2738-2742.

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

Wagner DD, Marder VJ. Biosynthesis of human von Willebrand protein by human endothelial cells; processing steps and their intracellular location. *J Cell Biol* 1984; 99:2123-2130.

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.

Titani K, Ericsson LH, Takio K, Kumar S, Chopek MW, Fuhikawa K. Chemical characterization of human von Willebrand factor. *Thromb Haemost* 1985; 54:123.

The VWF protein was sequenced.



Turitto VT, Weiss HJ, Zimmerman TS, Sussman II. Factor VIII/von Willebrand factor in subendothelium mediates platelet adhesion. *Blood* 1985; 65:823-831.

Vessel segments from rabbit aorta, denuded of their endothelial layer, contained significant quantities of FVIII/VWF in the subendothelial layer as determined by immuno-fluorescent microscopy. Denuded vessel segments were pre-incubated with goat antibodies to rabbit FVIII/VWF and then perfused with human blood at a high shear rate. Platelet adherence to the subendothelium was markedly reduced.

Stel HV, Sakariassen KS, de Groot PG, van Mourik JA, Sixma JJ. Von Willebrand factor in the vessel wall mediates platelet adherence. *Blood* 1985; 65:85-90.

Pre-treatment of normal vessel walls with monoclonal antibodies to sites on the VWF molecule inhibited adherence of platelets. Inhibition was shear-rate dependent, being significant at high shear-rates. VWF present in the vessel wall contributes to platelet adherence.

Moake JL, Turner HA, Stathopoulos NA, Nolasco LH, Hellum JD. Involvement of large plasma von Willebrand factor (VWF) multimers and unusually large VWF forms derived from endothelial cells in shear-stress induced platelet aggregation. *J Clin Invest* 1986; 78:1456-1461.

A fluid shear stress of 180 dyn/cm<sup>2</sup> 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 to GPIIb-IIIa.

Federici AB, Bader R, Pagani S, Colibretti ML, DeMarco L, Mannucci PM. Binding of von Willebrand factor to glycoproteins Ib and IIb/IIIa complex: affinity is related to multimeric size. *Brit J Haematol* 1989; 73: 93-99.

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.

De Marco L, Mazzucato M, De Roia D, Casonato A, Federici AB, Girolami A, Ruggeri ZM. Distinct abnormalities in the interaction of purified types IIA and IIB von Willebrand factor with the two platelet binding sites, glycoprotein complex Ib-IX and IIb-IIIa. *J Clin Invest* 1990; 86: 785-792.

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 and that of type 2B VWF was increased despite the absence of large multimers. Binding of both 2A and 2B VWF to platelet receptor GPIIb-IIIa was decreased.

Wise RJ, Dornier AJ, Krane M, Pittman DD, Kaufman RJ. The role of von Willebrand factor multimers and propeptide cleavage in binding and stabilization of factor VIII. *J Biol Chem* 1991; 266: 1948-1955.

Various mutant VWF molecules were made in a recombinant system. When the propeptide was deleted, a VWF dimer could form but multimers could not form. The dimer could not interact with platelet GPIb to mediate agglutination but could bind and stabilize FVIII. When the propeptide did not cleave off, large multimers did form and did function in platelet agglutination but could not bind and stabilize FVIII.

Alevriadou BR, Moake JL, Turner NA, Ruggeri ZM, Folie BJ, Phillips MD, Schreiber AB, Hrinca ME, McIntire LV. Real-time analysis of shear-dependent thrombus formation and its blockade by inhibitors of von Willebrand factor binding to platelets. *Blood* 1993; 81:1263-1276.

The precise sequence of interactions between VWF and platelet receptors GPIb and GPIIb/IIIa 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 insolubilized VWF. Aggregation occurred subsequently and required the binding of ligands including VWF...to GPIIb-IIIa."

Fressinaud E, Federici AB, Castaman G, Rothschild C, Rodeghiero F, Baumgartner HR, Mannucci PM, Meyer D. The role of platelet von Willebrand

factor in platelet adhesion and thrombus formation: a study of 34 patients with various subtypes of type I von Willebrand disease. *Br J Haematol* 1994; 86:327-332.

At a high shear rate, platelets from VWD patients with a normal platelet content of VWF (type 1 with normal platelet VWF and type 2 "Vicenza") adhered normally. At the same shear rate, platelets from VWD patients with type 1 VWD and low or dysfunctional platelet VWF had defective adhesion. Platelet VWF may substitute for plasma VWF to promote platelet adhesion.

Kulkarni S, Dopheide SM, Yap CL, Ravanat C, Freund M, Mangin P, Heel KA, Street A, Harper IS, Lanza F, Jackson SP. A revised model of platelet aggregation. *J Clin Invest* 2000; 105:783-791.

After studies of platelets with differing congenital deficiencies, in both *in vivo* and *in vitro* systems, the authors concluded that "platelet aggregation under flow appears to be a multi-step process involving: (a) exposure of VWF on the surface of immobilized platelets; (b) a reversible phase of platelet aggregation mediated by the binding of GPIb alpha on the surface of free-flowing platelets to VWF on the surface of immobilized platelets; and (c) an irreversible phase of aggregation dependent on integrin alpha (IIb)beta(3)" (GPIIb/IIIa). Platelet VWF has an important role in initiating aggregation under flow conditions.

Levy GG and many others. Mutations in a members of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. *Nature* 2001, 413:488-494.

In thrombotic thrombocytopenic purpura (TTP), proteolysis of VWF is decreased and unusually large multimeric forms of VWF are seen in the plasma. Seven families had congenital (recessive) TTP. A responsible gene locus was found on chromosome 9q34. A new member of the ADAMTS gene family was identified, "ADAMTS 13", a zinc metalloproteinase, and 12 different gene mutations identified. Heterozygotes had intermediate levels. ADAMTS13 deficiency is responsible for TTP.

Arya M, Anvari B, Romo GM, Dong JF, McIntire LV, Moake JL, Lopez JA. Ultra-large multimers of von Willebrand factor form spontaneously high-strength bonds with the platelet glycoprotein Ib-IX complex: studies using optical tweezers. *Blood* 2002; 99:3971-3977

The authors studied the force required to break the normal plasma VWF - platelet GPIb bonds induced by ristocetin and botrocetin. Ultra-large VWF multimers, as from TTP, bound to GPIb sites spontaneously (i.e. without ristocetin or botrocetin) 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 for binding in very large multimers than in smaller ones. The conformational state of VWF multimers may be critical to function. *The platelet site previously known simply as GPIb is further defined as GP Ib-IX-V or just GP Ib-IX in this and other recent articles.*

Tsai HM. Shear stress and von Willebrand factor in health and disease. *Semin Thromb Hemost* 2003; 29:479-488.

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, e.g. across a stenotic aortic valve or in small vessels in hemolytic-uremic disease, promote cleavage, with loss of the largest multimers.

Hollestelle MJ, Thinnis T, Crain K, Stiko A, Kruijt JK, van Berkel TJC, Loskutoff DJ, van Mourik JA. Tissue distribution of factor VIII gene expression *in vivo*: A closer look. *Thromb Haemost* 2001; 86:855-61.

Factor VIII gene expression (mRNA) was analyzed quantitatively in different tissues. Levels were high in liver and kidney, in hepatic sinusoidal endothelial cells and Kupffer cells (but not in hepatocytes) and also were high in renal glomeruli and tubular epithelial cells. VWF protein was predominantly located in the endothelium of larger vessels.

Eikenboom JCJ, Castaman G, Kamphuisen PW, Rosendaal FR, Bertina RM. The factor VIII/von Willebrand factor ratio discriminates between reduced synthesis and increased clearance of von Willebrand factor. *Thromb Haemost* 2002; 87:252-257.

Levels of FVIII and VWF normally are concordant. The level of

FVIII relative to VWF is increased if VWF synthesis is reduced. An increased level of FVIII relative to VWF 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. (The FVIII-VWF complex may be cleared without being dissociated.) 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 in those 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 VWF:Ag is very low, some unbound FVIII circulates and contributes notably to the total plasma FVIII.

Mendolicchio GL, Ruggeri ZM. New perspectives on von Willebrand factor functions in hemostasis and thrombosis. *Semin Hematol* 2005; 42:5-14. *Review*

### The VWF gene described

Ginsburg D, Handin RI, Bonthron DT, Conlon TA, Bruns GAP, Latt SA, Orkin SH. Human von Willebrand factor (vWF): Isolation of complementary DNA (cDNA) clones and chromosomal localization. *Science* 1985; 140:1-1406.

The gene for VWF was located on chromosome 12. Using cDNA, 618 basepairs were sequenced. (See also articles below)

Lynch DC, Zimmerman TS, Collins CJ, Brown M, Morin MJ, Ling EH, Livingston DM. Molecular cloning of cDNA for human von Willebrand factor: authentication by a new method. *Cell* 1985; 41:49-56.

Sadler JE, Shelton-Inloes BB, Sorace JM, Harlan JM, Titani K, Davie EW. Cloning and characterization of two cDNAs coding for human von Willebrand factor. *Proc Natl Acad Sci* 1985; 82:6394-6398.

Two clones were isolated, coding for more than 80% of the VWF molecule as it exists in plasma.

Verweij CL, DeVries CJM, Distel B, Van Zonneveld AJ, Van Kessel AG, Van Mourik JA, Pannekoek H. Construction of cDNA coding for human von Willebrand factor using antibody probes for colony-screening and mapping of the chromosomal gene. *Nucleic Acids Res* 1985; 13:4699-4717.

Shelton-Inloes BB, Titani K, Sadler JE. CDNA sequences for human von Willebrand factor reveal five types of repeated domains and five possible protein sequence polymorphisms. *Biochemistry* 1986; 25:3164-3171.

Domains of the VWF gene are defined.

Mancuso DJ, Tuley EA, Westfield LA, Worrall NK, Shelton-Inloes BB, Sorace JM, Alevy YG, Sadler JE. Structure of the gene for human von Willebrand factor. *J Biol Chem* 1989; 264: 19514-19527.

The cloned VWF gene is about 178 kilobases in length. The signal peptide and propeptide ("von Willebrand antigen II") are encoded by 17 exons in about 80 kilobases, and the mature subunit and 3' noncoding regions by 35 exons in the remaining approximately 100 kilobases. Given its size and number of exons (52) the VWF gene is the most complex of the genes encoding hemostatic proteins. "The highly repeated structure of human vWF suggests...several gene segment duplications." *The article quotes the entire nucleotide sequence.*

### Prevalence of VWD

Rodeghiero F, Castaman G, Dini E. Epidemiological investigation of the prevalence of von Willebrand's disease. *Blood* 1987; 69: 454-459

In Vicenza province, Italy, 1281 supposedly-normal children ages 11-14 were studied. A questionnaire asked about bleeding symptoms in the child, his siblings and his parents. 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." Separate adult normal ranges were established by testing 289 healthy adults. 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. Distribution of VWF:RCo values are described in the table below.

Subjects	N	Blood group	VWF:RCo, % mean	VWF:RCo, % range	VWF:RCo, % 2.5th percentile, lower limit
Children	510	O	99.7	37.4- 177.6	61.0
	656	Non-O	115.8	43.9- 230	77.0
Adults	121	O	88.7	46.0- 123.1	51.8
	168	Non-O	110.6	69.5-81.3	75.1

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. 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 the others whose levels of VWF:RCo range from 36 to 72%.) In these children, levels of VWF:RCo correlated with VWF:Ag and FVIII, and multimer analysis was normal, suggesting type 1 VWD. The approximate 1% prevalence of VWD has been widely quoted but also widely disputed. If only the single unquestionable case were considered, the prevalence would be 0.08%. Later, a VWF gene mutation was found in the child with obvious VWD, but not in the other children.

Werner E, Broxson E, Tucker EL, Giroux DS, Shults J, Abshire TC. Prevalence of von Willebrand disease in children: A multiethnic study. *J Pediatrics* 1993; 123:893-898.

VWF:RCo was measured in 600 healthy children ages 2-18 years seen for routine school physical examinations in the USA. Personal and family history of bleeding were determined by questionnaire. The 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 those criteria for VWD (seven with blood group O). Of these, one had indisputable VWD with a VWF:RCo level of 10% and multimers visible only after stimulation with DDAVP. 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 multimers. RIPA in three subjects was said to be diminished. A prevalence of VWD of 1.3% was claimed as a result of this study. (If only the unquestionable case were considered, the prevalence would be 0.16%.)

Tengborn L. Von Willebrand disease in Sweden: demography and treatment. *Haemophilia* 1999; 5(suppl 2), 73-76.

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 should be 27,000. (If heterozygotes were usually symptomatic, there would be many more patients with "type 1" VWD than the 586 known to exist.)

Scheibel E. Von Willebrand disease in Denmark: demography and treatment. *Haemophilia* 1999; 5: (suppl 2): 71.

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

Kekomaki R, Rasi V, Ebeling F, Vahtera E, Javela K, Koski T, Myllyla G, Ikkala E. *Haemophilia* 1999; 5 (suppl 2): 72-74.

In Finland, population about 5 million, 1076 patients with VWD were registered, including 695 type 1, 145 type 2 and 18 type 3. More women (450) than men (235) had type 1. No gender imbalance was seen in type 2 nor in diagnoses made in children. Type 2 VWD was much more likely to be diagnosed in childhood than was type 1 VWD. Only a small proportion of their patients originated from the Åland islands. The prevalence of symptomatic VWD was about 200 per million (one in 5000) and that of type 3 VWD was four in a million.

Mannucci PM, Bloom AL, Larrieu MJ, Nilsson IM, West RR. Atherosclerosis and von Willebrand factor. I. Prevalence of severe von Willebrand's disease in western Europe and Israel. *Br J Haematol* 1984; 57:163-169.

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 population), Norway (2.5), and Finland (2.2). The overall prevalence for Europe plus Israel was 0.45 per million.

### Variability of VWF in normal subjects

Buchanan GR, Holtkamp CA, Levy EN. Racial differences in ristocetin-induced platelet aggregation. *Br J Haematol* 1981; 49:455-464.

RIPA at the usual ristocetin level (1.1 mg/ml) in normal black subjects was, on average, less than half the amplitude seen in normal white subjects. The discrepancy was not explained by differences in age, sex, presence of sickle hemoglobin or medications. The white-black 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. Plasma from black subjects with absent aggregation, added to normal platelet-rich plasma from white subjects, inhibited aggregation.

Orstavik KH, Magnus P, Reisner H, Berg K, Graham JB, Nance W. Factor VIII and factor IX in a twin population. Evidence for a major effect of ABO locus on factor VIII level. *Am J Hum Genet* 1985; 37:89-101.

Twin sets were studied. The least variation of FVIII and factor IX was seen within the 74 sets of monozygotic twins, more variation within the 84 like-sexed dizygotic twins, and the most between unrelated pairs. Significantly higher levels of FVIII, VWF:Ag and factor IX were seen in older subjects (ages 57-62) than in younger ones (ages 33-39). No gender differences were seen. 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 (see table below). ABO group was not correlated with factor IX level. Of the total variance in VWF:Ag levels, about 30% was due to ABO blood group, and 66% to all genetic influences.

ABO group	N	Mean VWF:Ag, %, (translated from natural logarithms)
O	60	65
A-2	20	87
A-1	68	100
B	15	103
A-1/B	4	119

Gill JC, Endres-Brooks J, Bauer PJ, Marks Jr, WJ, Montgomery RR. The effect of ABO blood group on the diagnosis of von Willebrand disease. *Blood* 1987; 69:1691-1695.

Glycosylation levels vary according to blood groups, and VWF is highly glycosylated, thus VWF levels may reflect the variation in glycosylation associated with ABO groups. Among 142 patients previously diagnosed with VWD, type O was over-represented in type 1 patients (77% group O) but not in type 2 patients (31%). "There may be a subset of type 1 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. Similarly, individuals of blood group AB with a VWF genetic defect may have been overlooked because the diagnosis the vWF is elevated due to blood type." VWF:Ag and ABO

blood group was tested in 1117 blood donors in Milwaukee, with results as follows: (note the different lower limits of normal in different blood groups):

ABO type	N	VWF:Ag, %, geometric mean	Mean, ± 2 SD
O	456	74.8	35.6 - 157.0
A	340	105.9	48.0 - 233.9
B	196	116.9	56.8 - 241.0
AB	109	123.3	63.8 - 238.2

Caekbeke-Peerlinck K, Koster T, Briet E. Bleeding time, blood groups and von Willebrand factor. *Br J Haematol* 1989; 72:217-220.

A significantly longer bleeding time, by Duke and by Ivy methods, was found in normal people with blood group O (n=33) compared to people of other blood groups (n=37).

Blomback M, Eneroth P, Andersson O, Ancret M. On laboratory problems in diagnosing mild von Willebrand's disease. *Am J Hematol* 1992; 40:117-120.

Laboratory tests reflecting VWD were followed over one menstrual cycle in 15 normal young women. In three women, levels of VWF:RCO or of VWF:Ag sometimes fell below the normal range. Laboratory results in one woman were abnormal at the beginning of the menstrual cycle but within normal limits at the end. (Variation within the menstrual cycle has not been studied extensively; this report is often quoted.)

Shima M, Fujimura Y, Nishiyama T, Tsujiuchi T, Narita N, Matsui T, Titani K, Katayama M, Yamamoto F, Yoshioka A. ABO Blood group genotype and plasma von Willebrand factor in normal individuals. *Vox Sang* 1995;68:236-40.

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:

Harvey PJ, Keightley AM, Lam YM, Cameron C, Lillicrap D. A single nucleotide

Genotype	N	VWF:Ag, %, mean	VWF:RCo, %, mean
OO	68	80.9	82.2
AO	69	103.7	110.6
BO	52	100.5	107.3
AA	41	113.3	123.8
BB	31	114.5	125.3
AB	69	113.8	124.3

polymorphism at nucleotide -1793 in the von Willebrand factor (VWF) regulatory region is associated with plasma VWF:Ag levels. *Br J Haematol* 2000; 109:349-353

A single nucleotide polymorphisms (SNP) in VWF was in strong linkage disequilibrium with three previously reported SNPs and, like them, is significantly associated with plasma VWF levels. There were statistically-significant differences in mean VWF:Ag level according to possible genotypes G/G, G/C, and CC at this locus, as follows:

Genotype	N	VWF:Ag, %, mean
G/G	36	93
G/C	114	85
C/C	111	77

Miller CH, Dilley A, Richardson L, Hooper WC, Evatt BL. Population difference in von Willebrand factor levels affect the diagnosis of von Willebrand disease in African-American women. *Am J Hematol* 2001; 67:125-129.

Women with menorrhagia (70 black, 53 white) and age-matched controls were studied. Levels of VWF:Ag, VWF:RCo and FVIII were significantly higher in the non-O blood group than in the O blood group. Levels of VWF:Ag were significantly higher in group O blacks than in group-O whites. 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, ristocetin induced platelet aggregation (RIPA) was significantly lower in blacks than in whites. One black and seven white women with menorrhagia were classified as having VWD based on two test levels below two SD of the mean for their race and ABO group. (All "abnormalities" were mild.)

Miller CH, Haff E, Platt SJ, Rawlins P, Drews CD, Dilley AB, Evatt B. Measurement of von Willebrand factor activity: relative effects of ABO blood type and race. *J Thromb Haemost* 2003; 1:2191-2197.

Further tests on the 123 normal females in the above study included VWF:Ag, VWF:RCo, VWF:CB (collagen binding) and the monoclonal Ab test measuring VWF activity ("VWF:MoAB"). Whites had significantly lower levels in all tests, except VWF:RCo, than blacks. ABO blood group differences accounted for 19% of the total variance in VWF:Ag and race for 7% of the total variance. Results were as follows:

Race, ABO	VWF:Ag, % mean	VWF:RCo, % mean	VWF:CB, % mean	VWF:MoAb, % mean	FVIII, % mean
White, O	84	80	77	75	90
Black, O	104	80	98	83	100
White non-O	113	110	103	98	109
Black, non-O	140	112	128	132	132

When they re-examined the 123 women with menorrhagia mentioned in earlier paper, they found that the VWF:RCo was the best single discriminator between controls and symptomatic women. The authors said, "it is as yet unclear whether bleeding occurs in individuals who have VWF activity levels below a certain threshold or those whose levels fall below the norm for their group."

O'Donnell J, Boulton FE, Manning RA, Laffan MA. Genotype at the *Secretor* blood group locus is a determinant of plasma von Willebrand factor level. *Br J Haematol* 2002; 116:350-356.

*Secretor*, Lewis and ABO blood group genotypes and FVIII and VWF:Ag levels were measured in 136 normal persons. Lewis and *Secretor* types were determined with RFLP analysis (said to be more reliable than the serology used in earlier studies.) VWF:Ag and FVIII levels were slightly higher in persons with the A1A1 genotype compared to A1O1, and significantly higher in A1O1 than in either A2O1 or O1O1

When all ABO groups were considered together, significantly higher levels of VWF:Ag were found in persons homozygous for the *Secretor* allele (genotype Se-Se) than in heterozygotes (Se-se). In persons with O1O1 genotype, VWF:Ag levels were significantly higher in those with homozygous Se-Se genotype than in either heterozygotes or those homozygous for the null allele (se-se). In persons with A1O1 genotype, VWF:Ag levels also were significantly higher in Se-Se homozygotes compared to heterozygotes. Results are summarized in the following table:

ABO genotype	<i>Secretor</i> genotype	N	VWF:Ag, % mean	FVIII, % mean
O1O1	Se-Se	16	89.1	145
O1O1	Se-se	31	71.5	128
O1O1	se-se	14	72.8	124
A1O1	Se-Se	17	108.7	174
A1O1	Se-se	24	87.3	159
A1O1	se-se	12	102.6	177

Bowen DJ. An influence of ABO blood group on the rate of proteolysis on von Willebrand factor by ADAMTS13. *J Thromb Haemost* 2003; 1:33-40

VWF from persons of various blood groups was separated and subjected to proteolysis by ADAMTS13 over time. The rate of VWF proteolysis 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.

Bowen DJ, Collins PW. An amino acid polymorphism in von Willebrand factor correlates with increased susceptibility to proteolysis by ADAMTS13. *Blood* 2004; 103:941-947.

Increased susceptibility of VWF to proteolysis was linked to one allele of a single nucleotide polymorphism at position 24/1282 and may contribute to a type 1 VWD phenotype.

### Variability in VWD

Italian Working Group (Chair, PM Mannucci). Spectrum of von Willebrand's disease: a study of 100 cases. *Br J Haematol* 1977; 35: 101-112.

Italian patients with VWD were studied with six tests: Ivy BT, FVIII, VWF:Ag, VWF:RCo, RIPA, and platelet adhesion to glass beads. In 17 patients with severe VWD, all tests were abnormal; severe VWD appeared to be recessive. Moderate VWD, in 83 patients, appeared to be dominant. Nineteen patients with normal RIPA had mild to moderate deficiency of VWF:RCo and somewhat similar levels of FVIII and VWF:Ag; most had normal BTs (probably type 1). Among 52 patients with reduced RIPA, VWF:RCo was below the level of detection in 43 but levels of FVIII and VWF:Ag; BTs were normal to prolonged (probably type 2A). Twelve patients had increased RIPA, mildly reduced levels of FVIII, VWF:Ag and VWF:RCo and prolonged BTs (probably 2B). Patients soon were being classified according to multimer size distribution. See Ruggeri and Zimmerman, 1980, and Weiss et alia, 1983, on page 21., "History, Multimers".

Abildgaard CF, Suzuki Z, Harrison J, Jefcoat K, Zimmerman TS. Serial studies in von Willebrand's disease: Variability versus "variants". *Blood* 1980; 56:712-6.

Before this oft-quoted study, attempts were being made to classify milder VWD patients according to which tests (BT, FVIII, VWF:Ag, VWF:RCo) were in the normal range and which in the abnormal range, perhaps barely abnormal, with chaotic results. In this study, BT, FVIII, VWF:Ag and VWF:RCo were measured at intervals over two years in 50 patients with (mild) VWD from 25 families. Variation in results of a given test from time to time in the same patient was often seen, perhaps floating from the normal into the abnormal range and back. Types of VWD should not be based on patterns in these tests. (Diagnosis of mild VWD is difficult.)

Sadler JE, Matsushita T, Dong Z, Tuley EA, Westfield LA. Molecular mechanism and classification of von Willebrand disease. *Thromb Haemost* 1995; 74:161-166.

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.

De Romeuf C, Mazurier C. Comparison between von Willebrand factor (VWF) and VWF antigen II in normal individuals and patients with von Willebrand disease. *Thromb Haemost* 1998; 80:37-41.

In type 1 VWD, some patients had a parallel decrease in VWF:Ag and the propeptide VWF"Ag II and others had much lower VWF:Ag than propeptide. In type 2A patients who lack HMW VWF in platelets as well as in plasma, presumably due to deficient multimer formation ("group 1"), plasma levels of VWF:Ag and propeptide were decreased to a similar degree, whereas in type 2A patients who do have HMW VWF in platelets and who have increased proteolysis of VWF ("group 2"), the plasma level of propeptide was normal. In type 2B, the plasma level of propeptide was normal and higher than the level of VWF:Ag, as expected given that VWF is consumed by excessive interaction with platelets. The

finding that some type 1 VWD patients have normal propeptide levels and others have decreased levels suggests similar pathogenetic mechanisms as in groups 1 and 2 type 2A. In the following simplified table, the propeptide (Ag II) is related to VWF:Ag levels in different groups:

Group	Families N	Persons N	VWF:Ag, %mean	Ag II, % mean
Normal males		100	98	106.6
Type 1, concordant	2	2	41	46
Type 1, Ag II higher	5	6	16	54.5
Type 1, Ag II normal	2	7	6.4	82
Type 2A, concordant, "group 1"	2	3	28.6	26.6
Type 2A, Ag II higher, "group 2"	3	7	39.6	121
Type 2B	7	9	47	125

### Type 1 VWD

Peake O, Goodeve A. Type 1 von Willebrand disease. *J Thromb Haemost* 2007; 5(suppl 1): 7-11. An outstanding up-to-date review.

Miller CH, Graham JB, Goldin LR, Elston RC. Genetics of classic von Willebrand's disease. I. Phenotypic variation within families. *Blood* 1979; 54:117-136. (The families in this off-quoted, detailed study had type 1 VWD.)

Two large families, 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 persons who had apparently transmitted the disorder (having had an ancestor and a descendant with VWD) only 13 of 26, or half, had an abnormal laboratory test. The most-frequently-abnormal test was VWF:RCO; the least frequently abnormal was the BT.

Mannucci PM, Lombardi R, Bader R, Vianello C, Federici AB, Solinas M, Mazzucconi MG, Mariani G. Heterogeneity of type 1 von Willebrand disease: Evidence for a subgroup with an abnormal von Willebrand factor. *Blood* 1985; 66:796-802.

Seventeen patients from 13 families with type 1 VWD were classified according to the platelet content of VWF:Ag and VWF:RCO. In the platelet-discordant group, HMW multimers of VWF were present in the plasma but at a lesser concentration than low molecular weight forms. Their classification, and number of families, is as follows:

Type 1	Platelet VWF:Ag	Platelet VWF:RCO	BT	DDAVP response
platelet normal, 4 families	Normal	Normal	Minimally prolonged	Excellent, corrected VWF:Ag, VWF:RCO, BT
platelet low, 6 families	Low	Low	Very prolonged	Minimal response
platelet discordant, 3 families	Normal	Low	Very prolonged	Good for VWF:Ag, less for BT or VWF:RCO

Grainick HR, Rick ME, McKeown LP, Williams SB, Parker RI, Maisonneuve P, Jenneau C, Sultan Y. Platelet von Willebrand factor: An important determinant of the bleeding time in type 1 von Willebrand's disease. *Blood* 1986; 68:58-61.

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 amounts of 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.

D'Alessio P, Zwaginga JJ, de Boer HC, Federici AB, Rodeghiero F, Castaman G, Mariani G, Mannucci PM, deGroot PG, Sixma JJ. Platelet adhesion to collagen in subtypes of type 1 von Willebrand's disease is dependent on platelet von Willebrand factor. *Thromb Haemost* 1990; 64: 227-231

When whole blood was perfused over fibrillar collagen, platelet adhesion was reduced in type 1 patients with low, normal or discordant levels of platelet VWF:Ag and VWF:RCO, but was not as low as in type 3 VWD. When tested in another method, in which washed platelets were suspended in an albumin solution, adhesion was normal in platelet-normal type 1 VWD, decreased in platelet-discordant type 1 VWD, and lowest in platelet-low type 1 VWD (the latter level of adhesion was similar to that in severe VWD). The authors concluded that platelet VWF may contribute to platelet adhesion when plasma VWF is low.

Ewenstein BM, Inbal A, Pober JS, Handin RI. Molecular studies of von Willebrand disease: Reduced von Willebrand factor biosynthesis, storage, and release in endothelial cells derived from patients with type I von Willebrand's disease. *Blood* 1990; 75: 1466-1472.

Endothelial cells were cultured from umbilical veins of normal newborns and of 2 newborns with type 1 VWD. The VWD cells contained less mRNA encoding VWF and secreted less VWF than the normal cells.

Federici AB, De Groot PG, Moia M, Ijsseldijk MJW, Sixma JJ, Mannucci PM. Type I von Willebrand disease, subtype "platelet low"; decreased platelet adhesion can be explained by low synthesis of von Willebrand factor in endothelial cells. *Br J Haematol* 1993; 83:88-93.

Endothelial cells isolated from the umbilical vein of a newborn with type I platelet-low VWD released significantly less VWF than did normal endothelial cells. In addition, adhesion of normal platelets to the VWD umbilical artery was lower than to a normal umbilical artery. In type 1 platelet-low VWD, there also are low levels of VWF in endothelial cells and the subendothelium. Perhaps the variant is due to a reduced rate of production, storage and release of a normal VWF.

Eikenboom JCJ, Reitsma PH, Peerlinck KMJ, Briet E. Recessive inheritance of von Willebrand's disease type 1. *Lancet* 1993; 341:982-986.

In four unrelated families with type 1 VWD, a moderately-affected proband was shown to be a compound heterozygote for mutations in the VWF genes. Simple heterozygotes were unaffected or only mildly affected. (Beware of compound heterozygosity in a frequent disorder.)

Zhang Z, Lindstedt M, Blomback M, Anvret M. Effects of the mutant von Willebrand factor gene in von Willebrand disease. *Hum Genet* 1995; 96:388-394.

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. In persons with one of two certain mutations, 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 and usually associated with 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, the VWF:Ag range was 13% to 110%, with another mutation, the range was 12% to 94 %, and with a third, the range was 13 to 39%.

Eikenboom JCJ, Matshushita T, Reitsma PH, Tuley EA, Castaman G, Briet E, Sadler JE. Dominant type 1 von Willebrand disease caused by mutated cysteine residues in the D3 domain of von Willebrand factor. *Blood* 1996; 88:2433-2441.

A certain mutation in the VWF gene segregated with the phenotype of type 1 VWD in three members of a family. Affected persons had FVIII levels of 21-35%, VWF:Ag levels of 10-15% and VWF:RCO levels under 20% (the limit of detection), thus, the levels were reduced about equally. BTs were mildly to markedly prolonged. The mutant VWF gene was transfected into cells suitable for culture, a normal VWF gene was transfected into other such cells, and a mixture of normal and mutant genes were transfected into still other cells to mimic heterozygosity. The

cells transfected only with the mutant gene secreted VWF poorly, compared to normal. In the hybrid transfected cells, the mutant vWF caused a dose-dependent decrease in the secretion of VWF (that is, the mutant VWF was inhibitory, a "dominant-negative" effect).

Lethagen S., Frick K, Isaksson C, Kristoffersson A-C, Holmberg L. Revised classification and treatment of von Willebrand disease. (letter). *Thromb Haemost* 1998; 80:190-200

Many patients in Sweden, with prior diagnoses of type 1 VWD based on presence of multimers of all sizes, had notably lower levels of VWF:RCo than of VWF:Ag. These patients responded well to DDAVP. Using the new technique of densitometry to evaluate multimer sizes, it became clear that although large multimers were present, they were disproportionately reduced compared to smaller ones. These characteristics were shared by 72 patients from four families with one specific mutation and 11 patients from one family with a different mutation. Both mutations had been described in other countries as type 2 mutations. If more accurate laboratory testing leads to more re-classification, type 1 VWD might no longer be the predominant type of VWD. Some of these patients had been given DDAVP successfully for major surgery, but DDAVP might not have been used had they been thought to have type 2.

Coughlan TC, Blagg JL, Abulola M, Daly ME, Hampton KK, Makris M, Peake IR, Goodeve AC. Null alleles are not a common cause of type 1 von Willebrand disease in the British population. *Thromb Haemost* 1999; 82: 1373-1375.

Patients with type 3 VWD have two null alleles, that is, defective genes which result in no protein expression from that allele. Heterozygotes are expected to have a 50% reduction in qualitatively normal protein. Some heterozygotes are asymptomatic and some have a type 1 VWD phenotype. To estimate the frequency of type 1 VWD due to a null allele, the authors genotyped 36 unrelated type 1 patients and ruled out null alleles in 26 of them; they could not rule out the possibility in the others. The frequency of blood group O in their patients was 72%, compared to 43% in a control population.

Nitu-Whalley IC, Lee CA, Griffigen A, Jenkins PV, Pasi KJ. Type 1 von Willebrand disease: A clinical retrospective study of the diagnosis, the influence of the ABO blood group and the role of the bleeding history. *Br J Haematol* 2000; 108:259-264

Patients previously diagnosed with type 1 VWD (n=246) were reclassified into 'possible' VWD (having low levels of VWF activity by the monoclonal-Ab ELISA test, adjusted for the blood group, and a notable personal or family bleeding history) and 'definite' type 1 VWD (having low levels of VWF adjusted for the blood group, and a notable personal and a family bleeding history). On reclassification, only 144/246 patients had low VWF levels adjusted for blood group. All criteria for 'definite' type 1 VWD were met by 88/246, and 51/246 had "possible" type 1 VWD. A large proportion of patients, 102/246, remained an indeterminate group with blood group O, VWF levels between 35 and 50 % and a personal and/or family bleeding history. Bleeding tendencies were similar in patients of group O and non-O who had VWF levels between 35 and 50 %. Bleeding symptoms may depend on VWF levels regardless of ABO types.

Nitu-Whalley IC, Riddell A, Lee CA, Pasi KJ, Owens D, Enayat MS, Perkins SJ, Jenkins PV. Identification of type 2 von Willebrand disease in previously diagnosed type 1 patients: A reappraisal using phenotypes, genotypes and molecular modeling. *Thromb Haemost* 2000; 84:998-1004.

Of 317 patients registered with 1 VWD, previous multimer analysis had been performed on 111 of them and size distribution was normal. Thirty of these patients, from 17 unrelated families had a ratio of VWF activity / VWF:Ag of <0.7. RIPA was performed in 26 of these patients and was absent or decreased in all. Mutation analysis revealed eight different mutations in a total of nine kindreds. Four of these mutations previously had been described in type 2 VWD and four were novel. In the eight other eight kindreds, in whom no mutation was found, multimer size was analyzed with densitometric analysis (a more sensitive and objective way of measuring multimer size.) In five kindreds, multimer analysis showed multimers of all sizes in normal proportions. In three kindreds, HMW multimers were slightly decreased in quantity relative to lower weight multimers. In another kindred, ultra-large multimers were

seen, resembling type 2M Vicenza. Thus, with improved multimer analysis, some type 1 patients were re-classified as type 2.

Levy G, Ginsburg D. Getting at the variable expressivity of von Willebrand disease. *Thromb Haemost* 2001; 86:144-148.

Some 20% of variation in VWF level is attributable to ABO blood group, 40% to other genetic factors (not all of which are identified) and 40% to non-genetic issues such as age, stress and hormonal status. The spectrum of VWF levels in normal persons overlaps that in VWD. The VWF level is a complex trait whose genetic component involves a large number of genes each contributing only a minor individual effect.

Bodo I, Katsumi A, Tuley EA, Eikenboom JCJ, Dong Z, Sadler JE. Type I von Willebrand disease mutation Cys1149Arg causes intracellular retention and degradation of heterodimers: a possible general mechanism for dominant mutations of oligomeric proteins. *Blood* 2001, 98:2973-2979.

To study dimer formation in a highly-penetrant dominant, moderately severe type 1 VWD, a recombinant gene for VWF was made, lacking the A1 and/or A3 domains, as markers. All these forms of VWF were secreted efficiently as a full range of multimers. Co-transfection of a mutant gene (from an affected family) plus marker-recombinant VWF showed that 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 proteasomal degradation in the cytoplasm."

Casaña P, Martínez F, Haya S, Espinos C, Aznar JA. Significant linkage and non-linkage of type 1 von Willebrand disease to the von Willebrand factor gene. *Br J Haematol* 2001; 115:692-700.

In 12 families with definite or possible type 1 VWD, linkage of the phenotype with a VWF gene was confirmed in seven families and disproven in two. Studies were inconclusive in three families.

Sadler, JE. Von Willebrand disease type 1: a diagnosis in search of a disease. *Blood* 2003; 101: 2089-2093.

Some type 1 patients with mild symptoms have VWF levels that are low-normal or slightly below normal. The normal range of VWF is wide, the prevalence of mild bleeding symptoms in the general population is high, and the relationship between VWF level and bleeding is poor. Dr. Sadler estimates that 0.4% of the population will have bleeding symptoms and low VWF, plus a family history, just by chance. Population screening for bleeding symptoms and VWF levels may over-estimate prevalence of a genetic disease. Prevalence figures based on symptomatic patients referred to specialized centers estimate only 23 -113 cases of VWD per million population. The modest reduction in VWF usually seen in type 1 VWD confers only a modest risk of bleeding. Dr. Sadler would like to see a low VWF level regarded as a risk factor, not a disease.

O'Brien LA, James PD, Othman M, Berber E, Cameron C, Notley CRP, Hegadom CA, Sutherland JJ, Hough C, Rivard GE, O'Shaunessy D, Lillicrap D. Founder von Willebrand factor haplotype associated with type 1 von Willebrand disease. *Blood* 2003; 102:549-557

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 with incomplete penetrance.

Sadler JE, Rodeghiero F. Provisional criteria for the diagnosis of VWD type 1. *J Thromb Haemost* 2005; 3:755-777.

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".

Lanke E, Johansson AM, Hallden C, Lethagen C. Genetic analysis of 31 Swedish type 1 von Willebrand disease families reveals incomplete linkage to the von Willebrand factor gene and a high frequency of a certain disease haplotype. *J Thromb Haemost* 2005; 3:2656-2663.

**In 31 Swedish families with type 1 VWD, stringently diagnosed, the authors looked for linkage to some specific VWF gene (i.e., did everyone in a family who appeared to have VWD inherit the same VWF gene?) and found it in 27 families (87%) but not in four other families (13%). A possible common mutation was found in 6/27 families with genetic linkage. Blood group O was over-represented in the study group.**

Eikenboom J and many others. Linkage analysis in families diagnosed with type 1 von Willebrand disease in the European study, molecular and clinical markers for the diagnosis and management of type 1 VWD. *J Thromb Haemost* 2006; 4:774-782.

**Segregation analysis in 143 families with previously-diagnosed VWD suggested linkage in 70%. After exclusion of families with abnormal multimer patterns (by a highly sensitive test), the proportion of the remainder (those with normal multimers) in whom linkage could be demonstrated was only 46%. (In other words, in about a third of all the families studied, VWD was not related to one specific VWF gene. Their low levels of VWF and their bleeding symptoms may have multiple causes.)**

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.

**Mutations were identified in 105 of 150 index cases from families previously diagnosed with type 1 VWD (from the same group as above.) Mutations were found in 54 of 57 index patients who had slightly abnormal multimer structure on sensitive analysis; they might be re-classified as type 2. Mutations were found in 51 of 93 patients with normal multimer structure. Thus, only about a third of index patients had "true" type 1 VWD, i.e. a VWF gene mutation, not a mis-diagnosed form of type 2 VWD. Patients with VWF gene mutations, especially those with abnormal multimers, tended to have lower levels of VWF:Ag and VWF:RCO than other patients. Bleeding histories did not correlate with presence of a mutation. Eleven of 124 mutations were "null" mutations.**

James PD, Paterson AD, Notley C, Cameron C, Hegadorn C, Tinlin S, Brown C, O'Brien L, Leggo J. Genetic linkage and association analysis in type 1 von Willebrand disease: results from the Canadian type 1 VWD study. *J Thromb Haemost* 2006; 4:783-792.

**A study of 155 families with type 1 VWD in Canada demonstrated linkage to the VWF gene in only 41%.**

James PD, Notley C, Hegadorn C, Leggo J, Tuttle A, Tinlin S, Brown C, Andrews C, Labelle A, Chirinian Y, O'Brien L, Othman M, Rivard G, Rapson D, Hough C, Lillicrap D. The mutational spectrum of type 1 von Willebrand disease: results from a Canadian cohort study. *Blood* 2007; 109: 145-154.

**Mutations were found in 62% of 123 type 1 VWD index cases. The 50 different mutations were in a variety of locations in the gene. The likelihood of finding a mutation was correlated with lower VWF levels. Blood group O was over-represented in index patients with more than 30% VWF:Ag. Seven different mutations, found in 11 families, were associated with complete phenotypic penetrance.**

Cumming A, Grundy P, Keeney S, Lester W, Enayat S, Guilliat A, Bowen D, Pasi J, Keeling D, Hill F, Bolton-Maggs PH, Hay C, Collins P. An investigation of the von Willebrand factor genotype in UK patients diagnosed to have type 1 von Willebrand disease. *Thromb Haemost* 2006; 96:630-641.

**Among 32 UK families with the phenotype of type 1 VWD, mutations were found in only 53%. Linkage between the VWF gene and the type 1 phenotype was found in 13/32 families.**

#### Type 1 VWD and platelet dysfunction

Di Paola J, Federici AB, Mannucci PM, Canciani MT, Kritzik M, Kunicki TJ, Nugent D. Low platelet alpha2beta1 levels in type 1 von Willebrand disease correlate with impaired platelet function in a high shear stress system. *Blood*

1999; 93:3578-3582.

**The adhesion of flowing platelets to collagen depends not only on GPIIb-VWF interaction but also on collagen interaction with the platelet integrin alpha2 beta1 (GPIIb/IIIa). The gene for that integrin has at least three alleles, of which two are associated with low collagen-receptor density 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 alleles associated with low receptor density was significantly higher in type 1 VWD. In 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.**

Weiss HJ. The bleeding tendency in patients with low von Willebrand factor and type 1 phenotype is greater in the presence of impaired collagen-induced platelet aggregation. *J Thromb Haemost* 2004; 2:198-199.

**Dr. Weiss reviewed his own records of 87 VWD patients with VWF:Ag or VWF:RCO levels between 15 and 50%, and whose collagen-induced platelet aggregation had been tested, and scored their bleeding symptoms. Those with abnormal collagen-induced platelet aggregation tended to have had more bleeding problems. He wonders whether the common 807C allele within the alpha2 gene, associated with a low density of the alpha2beta1 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.**

Kunicki TJ, Federici AB, Salomon DR, Kozioł JA, Head SR, Mondala TS, Chisnar JD, Baronciani L, Canciani MT, Peake IR. An association of candidate gene haplotypes and bleeding severity in von Willebrand disease (VWD) type 1 pedigrees. *Blood* 2004; 104:2359-2367.

**The severity of bleeding symptoms in 14 unrelated VWD patients was graded, and DNA studied. Significantly increased or decreased bleeding scores were demonstrated in patients with certain haplotypes of three platelet glycoprotein genes with single nucleotide polymorphisms. Plasma levels of VWF, however, measured as VWF:RCO or VWF:Ag, had a stronger influence on bleeding scores than did glycoprotein haplotypes.**

Gudmundsdottir BR, Marder VJ, Orundarson PT. Risk of excessive bleeding associated with marginally low von Willebrand factor and mild platelet dysfunction. *J Thromb Haemost* 2006; 5:274-281.

**In Iceland, 809 teenagers responded to a questionnaire about bleeding symptoms. Of these, 63 had excessive bleeding (7.8%), defined as epistaxis six or more times a year, or bleeding from lacerations for more than 10 minutes, or bleeding after more than one surgical operation, or bleeding into muscles or joints without trauma, or menstrual bleeding for more than ten days. The most sensitive test, VWF:RCO, was in the lower fifth percentile of normal in 20.4% of those with excessive bleeding and in 5.4% of normal subjects. Decreased platelet responsiveness to both ADP and epinephrine was found in 12.8% of subjects with excess bleeding and 4.4% of normal subjects. Two individuals (0.3%) had both low VWF and platelet dysfunction.**

#### Type 2 VWD

Fressinaud E, Mazurier C, Meyer D. Molecular genetics of type 2 von Willebrand disease. *International J Haematol* 2002; 75:9-18. (Review, 95 refs).

**An outstanding exposition. The VWF subunit contains 4 types of homologous domains, which are arranged in the following order: D1-D2-D'-D3-A1-A2-A3-D4-B1-B2-B3-C1-C2-CK. The A1 domain contains binding sites of platelet GPIIb, botrocetin 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. Phenotype (type of VWD) corresponds to particular domains on the molecule, as shown in the table on the next page.**

Domain	Predominant type	Comments
D2	A rare type 2A	Decreased large multimers, increased proportion of protomers, decreased satellite bands, recessive inheritance. Mutation presumably interferes with VWF processing & multimer assembly.
D'	2N	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.
D3	(2N if close to D'), 2M Vicenza	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.
A1	2B	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.
A1	2M, 2A	Other mutations cause decreased affinity of VWF for platelet GPIb, with (2A) or without (2M) loss of HMW VWF.
A2	2A	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.
A3	New, no name	Mild disorder with decreased binding to collagen.
CK	2A	Absence of large multimers in plasma and platelets, abnormal internal structure of multimers seen with high resolution gels. Rare, dominant.

## Type 2A

### Typical type 2A

Firkin B, Firkin F, Stott L. Von Willebrand's disease type B: a newly defined bleeding diathesis. *Aust NZ J Med* 1973; 3:225-229.

A patient with bleeding problems had a prolonged BT but a normal FVIII. His platelet-rich plasma failed to aggregated with ristocetin (absent RIPA). When normal or hemophilic plasma was added to his platelet-rich plasma, it then could be aggregated by ristocetin. (Type 2A?)

Peake IR, Bloom AL, Giddings JC. Inherited variants of factor-VIII-related protein in von Willebrand's disease. *N Engl J Med* 1974; 291:113-117.

In most patients with VWD, levels of FVIII and of VWF:Ag are similarly deficient. Six patients with VWD from two families, however, had marked prolongation of BTs, normal or slightly-reduced FVIII and VWF:Ag levels but grossly impaired RIPA. On crossed-immunoelectrophoresis, VWF:Ag from these patients moved faster toward the anode than did normal VWF:Ag (Probable type 2 VWD with predominantly small multimers.)

Kernoff PBA, Gruson R, Rizza CR: A variant of factor VIII related antigen. *Br J Haematol* 1974; 26:435-440.

A patient with VWD had a prolonged BT, a low level of FVIII, and a normal level of VWF:Ag. On crossed-immunoelectrophoresis, VWF:Ag migrated faster than normal. (Another possible case of type 2A).

Sultan Y, Simeon J, Caen JP. Electrophoretic heterogeneity of normal factor VIII/von Willebrand protein, and abnormal electrophoretic mobility in patients with von Willebrand's disease. *J Lab Clin Med* 1976; 87:185-197.

In France, seven patients with VWD, from two families, had impaired RIPA. On crossed-immunoelectrophoresis, VWF:Ag migrated faster than normal.

Howard MA, Salem HH, Thomas KB, Hau L, Perkin J, Coghlan M, Firkin BG. Variant von Willebrand's disease type B—Revisited. *Blood* 1982; 60:1420-1428. (The authors used an old term for type 2A VWD)

Plasma VWD from a patient with type 2A VWD failed to react to ristocetin but did react to botrecetin, showing that the binding sites for the two reagents are different.

Hill FGH, Enayat MS, George AJ. Investigation including VIII:Ag multimeric analysis of a large kindred with type IIA von Willebrand's disease showing a dominant inheritance and similar gene expression in four generations. *Thromb Haemost* 1983; 50:723-739.

In one kindred, 13 persons in four generations had VWD. Inheritance was dominant and fairly penetrant. The BT was prolonged in 8/12 tested persons, RIPA was absent in 10 and reduced in two of 12 tested, and VWF:RC<sub>0</sub> was very deficient in all. VWF:Ag was within normal limits in eight patients and just below normal limits in five patients; FVIII was normal in two patients and below normal in eleven patients. Large multimers were absent in all patients. (Has figures of all multimers and crossed immunoelectrophoreses.)

Levene RB, Booyse FM, Chediak J, Zimmerman TS, Livingston DM, Lynch DC. Expression of abnormal von Willebrand factor from patient with type IIA von Willebrand disease. *Proc Natl Acad Sci* 1987; 84:6550-6554.

Endothelial cells were cultured from the umbilical vein of a newborn with type 2A VWD. The VWF produced by the cells had large multimers but in lesser amounts, relative to smaller multimers, than seen in normal umbilical vein cells. The largest 2A VWF multimers degraded more readily than normal multimers.

Dent JA, Berkowitz SD, Ware J, Kasper CK, Ruggeri ZM. Identification of a cleavage site directing the immunochemical detection of molecular abnormalities in type IIA von Willebrand factor. *Proc Soc Natl Acad Sci* 1990; 87:6306-10

A site in VWF was exceptionally vulnerable to cleavage in four of five patients with type 2A VWD.

Lyons SE, Bruck ME, Bowie EJW, Ginsburg D. Impaired intracellular transport produced by a subset of type IIA von Willebrand disease mutations. *J Biol Chem* 1992; 267: 4424-4430.

Seven different mutations, clustered in the A2 domain of the VWF gene, were responsible for type 2A VWD in nine of eleven kindreds studied. The pathogenetic mechanisms of these mutations differ. Three missense mutations impaired the transport of VWF multimers between the endoplasmic reticulum and the Golgi apparatus, with more profound effects on the secretion of HMW multimers than of smaller ones. Retention of HMW forms in the endoplasmic reticulum may inhibit production of adequate VWF under control of the patient's normal allele, that is, the mutant allele has a dominant-negative effect. Two other missense mutations allowed secretion of HMW multimers which appear to be excessively sensitive to extracellular proteases.

Haberichter SL, Ballistreri M, Christopherson P, Moratek P, Gavazova S, Bellissimo DB, Manco-Johnson MJ, Gill JC, Montgomery RR. Assay of the von Willebrand factor (VWF) propeptide to identify patients with type 1 von Willbrand disease with decreased VWF survival.

In four families with highly-penetrant, moderately severe VWD, due to two different mutations, the half-life of mature 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 in plasma distinguishes this pathophysiologic mechanism of type 1 VWD.

Hommais A, Stépanian A, Fressinaud E, Mazurier C, Meyer D, Girma JP, Ribba AS. Mutations C1157F and C1234W of von Willebrand factor cause intracellular retention with defective multimerization and secretion. *J Thromb Haemost* 2006; 4:148-157.

A patient with type 2A VWD and another with unclassified VWD had the above mutations in the D3 domain. Using site-directed mutagenesis and expression in mammalian cells, those mutations were shown to impair multimer formation and induce intracellular retention of VWF.



## Atypical 2A (types not included in 2A in 1984)

Ruggeri ZM, Nilsson IM, Lombardi R, Holmberg L, Zimmerman TS. Aberrant multimeric structure of von Willebrand factor in a new variant of von Willebrand's disease (Type IIC). *J Clin Invest* 1982; 70: 1124-1127

**A patient with VWD lacked the largest multimers. The internal structure of his multimers had a double-band pattern whereas normal multimers have a triplet band structure.**

Mannucci PM, Lombardi R, Pareti FI, Solinas S, Mazzucconi MG, Mariani G. A variant of von Willebrand's disease characterized by recessive inheritance and missing triplet structure of von Willebrand factor multimers. *Blood* 1983; 62:1000-1005.

**A child with VWD had a prolonged BT, very low VWF:RCo, and normal FVIII and VWF:Ag. VWF:Ag migrated rapidly on crossed-immunoelectrophoresis. No large multimers were seen. Using small-pore agarose for immuno-electrophoresis, her VWF:Ag multimers were shown to consist of a single rather than a triplet band.**

Kinoshita S, Harrison J, Lazerson J, Abildgaard CF. A new variant of dominant type II von Willebrand's disease with aberrant multimeric pattern of factor VIII-related antigen (Type IID). *Blood* 1984, 63:1369-1371.

**Two persons with VWD from one kindred had prolonged BTs, normal levels of FVIII and VWF:Ag but decreased VWF:RCo and absence of large multimers in plasma and platelets. With multimer analysis on high-resolution gels, a double band was seen, rather than the five bands seen with normal VWF and with 2A and 2B VWF.**

Ciavarella G, Ciavarella N, Antoncechci S, De Mattia D, Ranieri P, Dent J, Zimmerman TS, Ruggeri ZM. High-resolution analysis of von Willebrand factor multimeric composition defines a new variant of type I von Willebrand disease with aberrant structure but presence of all size multimers (type IC). *Blood* 1985; 66:1423-1429.

**Five patients with mild VWD from two kindreds had similar levels of FVIII and VWF:Ag and slightly lower levels of VWF:RCo. RIPA was mildly reduced. Multimers of all sizes were seen but the satellite bands were very faint. (These patients were later reclassified as type 2.)**

Hill FGH, Enayat MS, George AJ. Investigation of a kindred with a new autosomal dominantly inherited variant type von Willebrand's disease (possible type IID). *J Clin Pathol* 1985; 38:665-670.

**Five persons with VWD in one kindred had prolonged BTs, normal levels of FVIII and VWF:Ag, definitely reduced levels of VWF:RCo, and no large multimers. The internal structure of each multimer showed a single dense band rather than a triplet.**

Gralnick HR, Williams SB, McKeown LP, Maisonneuve P, Jenneau C, Sultan Y. A variant of type II von Willebrand disease with an abnormal triplet structure and discordant effects of protease inhibitors on plasma and platelet von Willebrand factor structure. *Am J Hematol* 1987; 24:259-266.

**A patient with VWD had a long BT, absent RIPA, reduced FVIII, reduced plasma and platelet VWF:Ag, and very low plasma and platelet VWF:RCo. Large and intermediate multimers of plasma and platelet VWF:Ag were absent. Migration of minor multimer bands had a different pattern than previously-described variants. This variant was called IIG.**

Federici AB, Mannucci PM, Lombardi R, Lattuada A, Colibretti ML, Dent JA, Zimmerman TS. Type IIC von Willebrand disease: New structural abnormality of plasma and platelet von Willebrand factor in a patient with prolonged bleeding time and borderline levels of ristocetin cofactor activity. *Am J Med* 1989; 32:2870-2893.

**A patient with VWD had a prolonged BT, normal levels of FVIII and VWF:Ag, a borderline level of VWF:RCo and reduced RIPA. Large multimers were absent from the plasma. With high-resolution agarose gels, a broad central band with a minor faster-moving satellite band was seen in plasma VWF multimers, whereas platelet multimers were doublets with a mobility pattern differing from any seen to date.**

Rabinowitz I, Tuley EA, Marcuso DJ, Randi AM, Firkin BG, Howard MA, Sadler JE. Von Willebrand disease type B: A missense mutation selectively abolishes

ristocetin-induced von Willebrand factor binding to platelet glycoprotein Ib. *Proc Natl Acad Sci* 1992; 89:9846-9849.

**Normally, VWF binding to platelet GPIb is induced *in vitro* by ristocetin or by botrocetin. In a patient (still called type B in Australia at the time, now an outdated term), who had multimers of all sizes, botrocetin-induced binding was normal but ristocetin-induced binding was absent. He had a missense mutation. A mutant recombinant protein expressed which had the same properties, thus, the two reagents, ristocetin and botrocetin, must act by different mechanisms**

Casonato A, Pontara E, Dannhauser D, Bertomoro A, Sartori MT, Girolami A. Type 1 Padua: A new variant of von Willebrand's disease. *Br J Haematol* 1992; 81: 615-617

**Four persons with VWD from one kindred had low VWF:Ag, VWF:RCo and FVIII, but normal RIPA. Multimers of all sizes were present; each multimer was composed of a doublet rather than a triplet structure.**

Ledford M, Rabinowitz I, Sadler JE, Kent JW, Covantos F. New variant of von Willebrand disease type II with markedly increased levels of von Willebrand factor antigen and dominant mode of inheritance: von Willebrand disease type IIC Miami. *Blood* 1993; 82:169-175.

**Six persons with VWD in three generations of one kindred had prolonged BTs, normal levels of FVIII, mildly-reduced to low-normal levels of VWF:RCo, and very high levels (>200%) of VWF:Ag in plasma. Multimer analysis of plasma showed loss of HMW multimers and increase of small multimers. Satellite bands were not seen, a pattern similar to type IIC. The platelets had normal levels of VWF:RCo and normal to slightly elevated levels of VWF:Ag; HMW multimers were present in platelets.**

Ribba AS, Christophe O, Derlon A, Cherei G, Siguret V, Lavergne JM, Girma JP, Meyer D, Pietu G. Discrepancy between IIA phenotype and IIB genotype in a patient with a variant of von Willebrand disease. *Blood* 1994; 83:833-841.

**A patient with "type 2A" VWD had a prolonged BT, very low VWF:RCo, decreased FVIII and VWF:Ag, absent large multimers and absent RIPA. He had mild thrombocytopenia from time to time. Ristocetin-induced binding of his VWF to platelet GPIb was reduced. The gene mutation, however, was typical of type 2B. His mutation was reproduced by site-directed mutagenesis. Multimerization of the resulting recombinant VWF (rVWF) within the cell was normal. The mutant rVWF was able to bind to platelet GPIb in the presence of ristocetin or even spontaneously. *In vitro*, the mutant VWF mimics 2B VWF, but it behaves like 2A VWF in the patient. (An example of frustrating genotype-phenotype non-correlation !)**

Gaucher C, de Romeuf C, Rauis-Morre M, Corazza F, Fondu P, Mazurier C. Diagnosis of subtype 2B von Willebrand disease in a patient with 2A phenotype of plasma von Willebrand factor. *Thromb Haemost* 1995; 73: 610-616.

**A woman with "type 2A" VWD lacked large multimers in plasma. RIPA was not enhanced. Her plasma VWF had no increased capacity to bind to normal platelets in the presence of low ristocetin concentrations. The mutation found in her gene had previously been found in several families with typical type 2B VWD.**

Meyer D, Fressinaud E, Hilbert L, Ribba AS, Lavergne JM, Mazurier C. Type 2 von Willebrand disease causing defective von Willebrand factor-dependent platelet function. *Best Pract Res Clin Haematol* 2001; 14:349-364.

**Nowadays, type 2 VWD is being identified primarily according to the gene mutation. (If available!)**

## Type 2B

Rivard GE, Daviault MB, Brault N, D'Aragnon L, Raymond R. Von Willebrand's disease associated with thrombocytopenia and a fast migrating factor VIII related antigen. *Thromb Res* 1977; 2:507-516.

**Ten persons in a three-generation kindred had dominantly-inherited VWD. Five tested had prolonged BTs, intermittent mild thrombocytopenia, reduced FVIII levels and mildly reduced VWF:Ag. On crossed immunoelectrophoresis, VWF:Ag moved faster than normal. RIPA with the usual concentrations of ristocetin was normal. (Low levels of ristocetin were not tried but these patients may have had 2B, and this may be the first description.)**

Takahashi H, Sakuragawa N, Shibata A. Von Willebrand disease with an increased ristocetin-induced platelet aggregation and a qualitative abnormality of the factor VIII protein. *Am J Hematol* 1980; 8:299-308.

Two related persons with VWD had prolonged BTs, low-normal to slightly reduced levels of FVIII and VWF:Ag, and definitely reduced levels of VWF:RCO. On crossed-immunoelectrophoresis, VWF:Ag moved faster than normal. Platelet-rich plasma aggregated with lower concentrations of ristocetin than normal. Washed platelets from one of the patients had increased binding affinity to normal FVIII-VWF complex compared to normal washed platelets. (A full, clear description of type 2B VWD).

Ruggeri ZM, Pareti FI, Mannucci PM, Ciavarella N, Zimmerman TS. Heightened interaction between platelets and factor VIII/von Willebrand factor in a new subtype of von Willebrand's disease. *N Engl J Med* 1980; 302:1047-1051

In 20 persons with VWD from five families, VWF binding to platelets in the presence of ristocetin was enhanced; the condition was called "type IIB" (2B). VWF:RCO levels tended to be low but were not as low as those of eight patients with type 2A VWD. BTs were sometimes long but not as often as in type 2A. (Another clear description of type 2B)

Ruggeri ZM, Lombardi R, Gatti L, Bader R, Valsecchi C, Zimmerman TS. Type IIB von Willebrand's disease: Differential clearance of endogenous versus transfused large multimer von Willebrand factor. *Blood* 1982; 60:1453-1456.

After infusion of DDAVP into a patient with type 2B VWD, large multimers were released into the plasma but started disappearing early, at two hours. After infusion of normal cryoprecipitate (a source of normal VWF), large multimers still could be seen at seven hours. "The characteristic absence of larger molecular forms of VWF from IIB plasma is related to rapid removal of an intrinsically abnormal molecule after release from tissue stores."

Holmberg L, Nilsson IM, Borge L, Gunnarsson M, Sjorin E. Platelet aggregation induced by 1-desamino-8-D-arginine vasopressin (DDAVP) in type IIB von Willebrand's disease. *N Engl J Med* 1983; 309:816-821.

Infusion of DDAVP caused platelet aggregation *in vivo*, with thrombocytopenia, in patients with type 2B VWD. If post-DDAVP platelet-poor plasma (containing fresh, HMW VWF excessively avid for platelet GP1b) from a patient with type 2B VWD was added to normal platelet-rich plasma, the platelets aggregated. Large multimers were adsorbed onto the platelets. The authors warned against the use of DDAVP in type 2B VWD (This proscription later was reconsidered, see section on DDAVP.)

Weiss HJ, Sussman II. A new von Willebrand variant (Type I, New York): increased ristocetin-induced platelet aggregation and plasma von Willebrand factor containing the full range of multimers. *Blood* 1986; 68:149-156.

Three persons with VWD in one kindred in New York had borderline or slightly low levels of FVIII, VWF:Ag and VWF:RCO. RIPA was enhanced. Multimers of all sizes were present, a pattern indistinguishable from type 1 VWD. (This condition later was reclassified with 2B.)

Holmberg L, Berntorp E, Donner M, Nilsson IM. Von Willebrand's disease characterized by increased ristocetin sensitivity and the presence of all von Willebrand factor multimers in plasma. *Blood* 1986; 68:668-672.

Eight patients with VWD in one kindred in Malmö, Sweden, had normal to mildly reduced levels of FVIII, VWF:RCO and VWF:Ag and enhanced RIPA. All multimers were present in the plasma. No thrombocytopenia occurred after DDAVP infusion. Post-DDAVP patient plasma did not aggregate normal platelets. This phenotype was similar to that called "type I New York" (and was called "type Malmö"; both now under 2B.)

Wyllie B, Gibson G, Uhr E, Kronenberg H. Von Willebrand's disease characterized by increased ristocetin sensitivity and the presence of all von Willebrand factor multimers in plasma: A new subtype. *Pathology* 1988; 20:62-63.

A man with VWD had a normal level of FVIII and reduced levels of VWF:Ag and VWF:RCO. RIPA was enhanced. Multimers of all sizes, however, were seen on immunoelectrophoresis of his VWF:Ag.

Rick ME, Williams SB, Sacher RA, McKeown LP. Thrombocytopenia associated with pregnancy in a patient with type IIB von Willebrand's disease. *Blood* 1987; 69:786-789.

A woman with type 2B VWD became thrombocytopenic, with platelet counts of 20,000 to 30,000/cu mm, during each of two pregnancies. An increase of intermediate-sized multimers was seen. Type 2B VWD should be considered if a pregnant woman develops thrombocytopenia

Donner M, Holmberg I, Nilsson IM. Type IIB von Willebrand's disease with probable autosomal recessive inheritance and presenting as thrombocytopenia in infancy. *Br J Haematol* 1987; 66: 349-354.

Three babies who presented with thrombocytopenia, from two kindreds, were found to have type 2B VWD. Parents and other relatives had no bleeding problems or laboratory stigmata of VWD.

De Groot PG, Federici AB, De Boer HC, D'Alessio P, Mannucci PM, Sixma JJ. Von Willebrand factor synthesized by endothelial cells from a patient with type II B von Willebrand disease supports platelet adhesion normally but has an increased affinity for platelets. *Proc Natl Acad Sci USA* 1989; 86:3793-3797.

Endothelial cells were isolated from the umbilical vein of a newborn with type 2B VWD and a normal newborn. The synthesis, storage, secretion and multimer size distribution of VWF in the patient's endothelial cells was indistinguishable from normal. VWF secreted from patient cells bound to platelets at concentrations of ristocetin lower than those necessary for VWF from normal cells. Type 2B VWF stored in Weibel-Palade bodies was released upon stimulation with phorbol ester and bound almost completely to platelets in the absence of ristocetin. "These data support the hypothesis that the absence of highly multimeric forms of VWF in plasma of type IIB VWD is due to specific removal of these multimers by platelets."... "Normal VWF must first adsorb to a surface or interact with ristocetin before it can bind to platelet membrane glycoprotein Ib. In contrast, IIB VWD secretes vWF that is already in a form that can react directly with platelets."

Sweeney JD, Hoernig LA, Behrens AH, Novak E, Swank RT. Von Willebrand's variant (type II Buffalo): Thrombocytopenia after desmopressin but absence of *in vitro* hypersensitivity to ristocetin. *Am J Clin Path* 1990;93: 522-525.

Three persons with VWD in one kindred had mild deficiencies of FVIII and more severe deficiencies of VWF:Ag and VWF:RCO. Large multimers were absent. RIPA was absent. These findings suggest type 2A, but, after DDAVP infusion, platelet counts fell, with visible agglutination, from 148,000/cu mm to 18,000 in one patient and from 281,000 to 97,000 in another. Levels of FVIII, but not VWF:Ag, rose after DDAVP.

Hultin MB, Sussman II. Postoperative thrombocytopenia in type IIB von Willebrand's disease. *Am J Hematol* 1990; 33:64-68.

Platelet counts fell after surgical operations in members of a large kindred with type 2B VWD. In one patient, the platelet count fell from 144,000/cu mm to 23,000/cu mm with clumping on blood smears. Three other patients had less striking post-operative falls in platelet counts. Multimers of larger-than-usual sizes were found in the plasma post-operatively. The authors postulate that stress released VWF into the circulation and the fresh, HMW multimers aggregated platelets.

Donner M, Kristoffersson AC, Lenk H, Scheibel E, Dahlback B, Nilsson IM, Holmberg IM. Type IIB von Willebrand's disease: Gene mutations and clinical presentation in nine families from Denmark, Germany and Sweden. *Br J Haematol* 1992; 82:58-65.

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

Test	N	Range	Median, extrapolated
FVIII	12	22-64%	42%
VWF:Ag	12	32-68%	37%
VWF:RCO	11	5-33%	10%
VWF:CB	11	4-42%	12%

Holmberg L, Dent A, Schneppenheim R, Budde U, Ware J, Ruggeri ZM. Von Willebrand factor mutation enhancing interaction with platelets in patients with normal multimeric structure. *J Clin Invest* 1993; 91: 2169-2177

Four patients with VWD from three families, with the same mutation, had enhanced RIPA but normal VWF multimer distribution in plasma (formerly called "type I New York"). The isolated domain of VWF that binds to GB1b was expressed as a recombinant peptide from normal and from mutant VWF. The mutant domain supported platelet aggregation with lower concentrations of ristocetin than did the normal domain.

Facey DA, Favalaro EJ, Maxwell E, Baker R, Hertzberg MS. Type 2B von Willebrand's disease in thirteen individuals from five unrelated Australian families: Phenotype and genotype correlations. *Am J Hematol* 2000; 63:197-199.

In type 2B VWD, VWF has increased affinity for the platelet glycoprotein Ib-IX-V (GPIb) receptor complex. There is spontaneous binding of type 2B VWF to platelets and subsequent clearance of large multimers, and, sometimes, platelets. In all the affected persons in this survey in Australia, RIPA was enhanced and multimer analysis showed reduced HMW VWF. Plasma factor levels were as follows:

Test	N	Range	Median
FVIII	10	9-31%	14%
VWF:Ag	10	6-16%	9.5%
VWF:RCo	10	6-13%	8%

Ruggeri ZM. Type IIB von Willebrand disease: a paradox explains how von Willebrand factor works. *J Thromb Haemost* 2004; 2:2-6. *Historical review*

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." (*Ristocetin is present in high concentration in the ristocetin co-factor test, and in lower concentrations, showing a threshold of response, in RIPA. The ristocetin cofactor test typically is low in type 2B although RIPA is enhanced.*) "...the main reason why large VWF multimers and platelets can coexist in circulating blood with minimal interference may rest in the rapidly reversible nature of the VWF:GPIb $\alpha$  bond." The mutations in type 2B VWD "...increase the 'affinity' of binding to GPIb even though the interaction is still reversible under flow conditions." "This enhanced binding...is sufficient to favor the accumulation of VWF on the platelet surface; as a consequence, ...the largest, most adhesive multimers are removed from the circulation." "The largest multimers in plasma are clearly necessary for normal hemostasis and if they are absent, smaller multimers cannot compensate even if they contain a gain-of-function mutation."

Szanto T, Schlammadinger A, Salles I, Pareyn I, Vauterin S, Harsfalvi J, Vanden Brucke AM, Deckmyn Vanhourelbeke K. Type 2B von Willebrand disease in seven individuals from three different families: Phenotypic and genotypic characterization. *Thromb Haemost* 2007; 98:251-254.

Six of seven patients, from two unrelated families, had the same R1306W mutation but there was great variation in laboratory phenotypes. Bleeding times ranged from normal to prolonged, platelet counts and VWF:RCo from very low to normal, and FVIII:C and VWF:Ag from mildly deficient to normal. Of these laboratory tests, different ones were the most abnormal in different subjects. All subjects had increased aggregation of platelets with ristocetin and all (except one on whom a full history was not available) had excessive bleeding. The variable expression of a single genotype was striking.

Rayes J, Hommais A, Legendre P, Tout H, Veyradier A, Obert B, Ribba AS, Girma JP. Effect of von Willebrand disease type 2B and type 2M mutations on the susceptibility of von Willebrand factor to ADAMTS-13. *J Thromb Haemost* 2006; 5:323-28.

Recombinant ADAMTS-13 was used to digest various recombinant full-length forms of VWF carrying molecular abnormalities identified in two type 2A patients, four type 2B patients and five type 2M patients. All mutant VWF showed some increased sensitivity to ADAMTS-13 with 2A having the greatest and 2B the next-greatest sensitivity. Thus, in type

2B, part of the reason for absence of large multimers is increased sensitivity to ADAMTS-13.

## Type 2M

Mannucci PM, Lombardi R, Castaman G, Dent JA, Lattuada A, Rodeghiero F, Zimmerman TS. Von Willebrand disease "Vicenza" with larger-than-normal (supranormal) von Willebrand factor multimers. *Blood* 1988; 71:65-70

Ten patients with VWD from two unrelated families in Vicenza province, Italy, had mild bleeding problems inherited in autosomal dominant style. Larger-than-normal VWF multimers, similar to those seen in normal persons immediately after DDAVP infusion, 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 follows:

Test	N	Range, %	Median
FVIII	7	15.1-45.0	20
VWF:Ag	7	6.25-32.0	14
VWF:RCo	7	7.1-29.6	11.1
VWF:CB	5	7.15-29.0	13.5

Zieger B, Budde U, Jessat U, Zimmerman R, Simon M, Katzel R, Sutor AH. New families with von Willebrand disease type 2M (Vicenza). *Thromb Res* 1997;87:57-64

Thirteen patients in seven unrelated families in Germany had type 2M VWD with super-large VWF multimers in the plasma. Levels of FVIII, VWF:Ag, VWF:RCo and VWF:CB were reduced. In some families, platelet VWF was normal and in others it was low. Inheritance was autosomal dominant.

Casonato A, Pontara E, Sartorello F, Bertomoro A, Durante C, Girolami A. Type 2M von Willebrand disease variant characterized by abnormal von Willebrand factor multimerization. *J Lab Clin Med* 2001;137: 70-76.

A mother and son had VWD with low plasma and platelet VWF, low FVIII, lower levels of VWF:RCo, almost absent RIPA, and impaired ristocetin-induced VWF binding to GPIb. HMW multimers were seen but the multimer structure was a "diffuse smear" instead of bands and satellite bands. After DDAVP, unusually large multimers appeared, larger than those seen from human umbilical vein endothelial cells.

Casonato A, Pontara E, Sartorello F, Cattini MG, Sartori MT, Padriani R, Girolami A. Reduced von Willebrand factor survival in type Vicenza von Willebrand disease. *Blood* 2002; 99:180-184.

Four additional families are described, who, unlike the original ones, have normal RIPA. After DDAVP, plasma levels of VWF and FVIII rise, but disappear rapidly, with return to baseline by four hours after infusion. The authors postulate normal synthesis but reduced survival of VWF in this group. BTs were normal in 4/7 tested patients.

Riddell AF, Jenkins PV, Nitu-Whalley IC, McCraw AH. Use of the collagen-binding assay for von Willebrand factor in the analysis of type 2M von Willebrand disease: a comparison with the ristocetin cofactor assay. *Br J Haematol* 2002; 116:187-192.

The utility of VWF:RCo and VWF:CB, for differential diagnosis were compared in patients with 2M, 2A and 2B VWD. VWF:CB was deficient in 2A and 2B, in which there is loss of HMW multimers, but was less deficient in type 2M, in which there are normal multimers. VWF:CB may be a useful test to distinguish 2M from other type 2 variants. Mean test results were as follows:

Type	N	VWF:Ag, %	VWF:RCo, %	VWF:CB, %
Normal	22	97	92	98.5
2A	6	22.5	< 5	5
2B	1	32	17	5
2M	25	27	5	35

Ribba AS, Loisel I, Lavergne JM, Juhan-Vague I, Obert B, Cherel G, Meyer D, Girma JP. 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 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.)

### Type 2N

Mazurier C. Von Willebrand disease masquerading as haemophilia A. *Thromb Haemost* 1992; 67:391-396. Review, 30 refs.

In eight patients with type 2N VWD, bleeding symptoms were typical of VWD including nosebleeds, menorrhagia, bruising and post-surgical bleeding. Three 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, FVIII levels 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:

Test	N	Range	Median
FVIII (activity)	8	5-22 %	About 7.5 %
FVIII:Ag	6	5.5-27.5 %	About 7.5 %
VWF:Ag	8	55-150%	100 %
VWF:RCo	8	65-100%	About 100 %

Lavergne JM, Piao Y, Ribba AS, Girma JP, Siguret V, Pietu G, Boyer-Neumann C, Schandelong A, Bahnak BR, Meyer DM. Functional analysis of the Arg91Gln substitution in the factor VIII binding domain of von Willebrand factor demonstrates variable phenotypic expression. *Thromb Haemost* 1993; 70:691-696.

Homozygotes and heterozygotes for VWD type 2N, all with the same mutation, from four families, were studied. Levels of VWF binding to FVIII were severely defective in homozygotes and half-normal in heterozygotes in two families. In a third family, a woman heterozygous for a 2N mutation had severely defective binding; her daughter had type 1 VWD with normal binding and her heterozygous son had half-normal VWF-FVIII binding. The mother may have been a compound heterozygote for VWD types 2N and 1, expressing primarily type 2N VWF. A heterozygote for the 2N mutation from the fourth family had borderline-normal FVIII, reduced VWF:Ag and VWF:RCo, and normal VWF-FVIII binding; presumably, she expresses primarily the VWF coded by her other (non-2N) gene, causing a type 1 phenotype. (Suspect compound heterozygosity in atypical cases.)

Schneppenheim R, Budde U, Krey S, Drewke E, Bergmann F, Lechler E, Oldenburg J, Schwaab R. Results of screening for von Willebrand disease type 2N in patients with suspected haemophilia A or von Willebrand disease type 1. *Thromb Haemost* 1996; 76:598-602

Using a test for VWF binding to FVIII, type 2N VWD was detected in five of 177 unrelated patients previously thought to have hemophilia A and three of 199 unrelated patients previously thought to have type 1 VWD. The R91Q mutation, already described in type 2N VWD, was found as a homozygous disorder in four unrelated patients. Compound heterozygosity of R91Q with a null mutation resulted in a very low level of binding of VWF to FVIII plus a borderline or mildly deficient level of VWF:Ag. In one instance, a compound heterozygote had a severe phenotype with FVIII levels of 1-2%.

Mazurier C, Parquet-Gomez A, Gaucher C, Lavergne JM, Goudemand J. Factor VIII deficiency not induced by FVIII gene mutation in a female first cousin of two brothers with haemophilia A. *Br J Haematol* 2002; 119: 390-392.

A young woman, maternal first cousin of brothers with hemophilia A, had a low FVIII level (varying from 4 to 10%) and a low-normal

level of VWF:Ag (30-53 %) and, at first, was presumed to be a hemophilia carrier. The brothers were found to have an inversion mutation which was not present in the young woman. Instead, she was a compound heterozygote for type 1 and 2N VWF mutations. She had a severe defect in VWF-FVIII binding. Her mother, two sisters, and a maternal aunt had her 2N mutation and intermediate levels of binding. Her father, who carried her other mutation, had FVIII and VWF levels at the lower end of the normal range and normal VWF:FVIII binding. A maternal aunt, mother of the hemophilic boys, who had their inversion mutation on her FVIII gene and the 2N mutation on a VWF gene, had FVIII levels ranging from 10-26% and VWF:Ag levels of 42-100. (It is dangerous to assume that persons in a given kindred, with similar phenotypes, have the same genotype.)

Casonato A, Sartorello F, Cattini MG, Pontara E, Soldera C, Bertomoro A, Girolami A. An Arg760Cys mutation in the consensus sequence of the von Willebrand factor propeptide cleavage site is responsible for a new von Willebrand disease variant. *Blood* 2003; 101:151-156.

In two related persons with VWD, the VWF propeptide did not cleave from multimers. The patients had mild deficiencies or borderline levels of FVIII, VWF:Ag, VWF:RCo and VWF:CB but definite deficiencies of VWF binding to FVIII. The mutant VWF and normal VWF were expressed together in recombinant cells, resulting in decreased VWF secretion, persistence of the propeptide and a defect in FVIII binding of VWF.

### Type 3

Shoa'i I, Lavergne JM, Ardaillou N, Obert B, Ala F, Meyer D. Heterogeneity of von Willebrand's disease: Study of 40 Italian cases. *Br J Haematol* 1977; 33:67-83.

Severe VWD was found in 22 patients from 11 families. Consanguinity was documented in ten families. Parents were asymptomatic but had levels of VWF:Ag reduced to about half-normal levels and of VWF:RCo to about quarter-normal levels. (Thus, some asymptomatic heterozygotes for null alleles might be diagnosed as having mild VWD if their mild deficiencies of VWF:RCo and borderline VWF:Ag were detected.)

Zimmerman TS, Abildgaard CF, Meyer D. The factor VIII abnormality in severe von Willebrand's disease. *New Engl J Med* 1979; 301: 1307-1310.

Using radio-labeled antibody, traces of VWF:Ag could be detected in seven unrelated patients with VWF:Ag <1% and another patient with VWF:Ag of 6%. On crossed-immunoelectrophoresis, five patients had abnormal patterns. Two lacked slower-moving (HMW) forms and three had a relative decrease in slower-moving forms. All their parents had normal levels of FVIII; two had average-normal levels of VWF:Ag and ten had about half-normal levels. VWF:Ag from all parents migrated normally on crossed-immunoelectrophoresis.

Berliner SA, Selgsohn U, Zivelin A, Zwang E, Soffer G. A relatively high frequency of severe (type III) von Willebrand's disease in Israel. *Br J Hematol* 1986; 62:535-543.

Eight of 16 patients with type 3 VWD were Arabs, i.e. a high frequency among Arabs of 5.3 per million (perhaps due to the high rate of consanguinity.) In 15 presumed heterozygotes, mean levels of FVIII, VWF:Ag and VWF:RCo were significantly higher than mean levels in type 1 VWD but also significantly lower than in normal persons.

Shelton-Inloes BB, Chehab FF, Mannucci PM, Federici AB, Sadler JE. Gene deletions correlate with the development of alloantibodies in von Willebrand disease. *J Clin Invest* 1987; 79:1459-1465.

Alloantibodies to VWF have been described only in patients with type 3 VWD. Two unrelated patients with type 3 VWD, among 19 studied, had large gene deletions and also had inhibitory alloantibodies.

Mannucci PM, Lattuada A, Castaman G, Lombardi R, Colibretti ML, Ciavarella N, Rodeghiero F. Heterozygous phenotypes of platelet and plasma von Willebrand factor in obligatory heterozygotes for severe von Willebrand's disease. *Blood* 1989; 74:2433-2436.

Presumed heterozygotes (n=28) in 15 families with severe VWD were studied. Levels of VWF:Ag and VWF:RCo were reduced in both

plasma and platelets in 11 persons, in platelets but not plasma in 8 persons, in plasma but not platelets in 5 persons, were normal in both plasma and platelets in one person and were unclassifiable in three persons. (Assignments into these categories sometimes rested on minor deficiencies, in other persons, discrepancies were striking.) The seven heterozygotes with prolonged BTs had the plasma low-platelet low phenotype. The different patterns were presumed due to different mutations.

Peake IR, Liddell MB, Moodie P, Standen G, Mancuso DJ, Tuley EA, Westfield LA, Sorace JM, Sadler JE, Verweij CL, Bloom AL. Severe type III von Willebrand's disease caused by deletion of exon 42 of the von Willebrand factor gene: Family studies that identify carriers of the condition and a compound heterozygous individual. *Blood* 1990; 75:654-661.

Seven heterozygotes related to a homozygous patient with a partial VWF gene deletion were studied. Six heterozygotes were asymptomatic, with normal levels of VWF in five and a mild deficiency in the sixth. The seventh heterozygote was moderately affected with 9% FVIII and 3% VWF:Ag and VWF:RCO, and was presumed to be a compound heterozygote, inheriting the deletion mutation from his father and a recessive mutation, not identified, from his phenotypically normal mother.

Eikenboom JCJ, Ploos Van Amstel HK, Reitsma PH, Briet E. Mutations in severe, type III von Willebrand's disease in the Dutch population: Candidate missense and nonsense mutations associated with reduced levels of von Willebrand factor messenger RNA. *Thromb Haemost* 1992; 68:448-454.

Two mutations were seen repeatedly in severe VWD (but in none of the type 1 VWD patients studied). Three of nine patients with type 3 VWD were homozygous for a specific single nucleotide substitution. Another three of the nine were heterozygous for a specific nonsense mutation. (Perhaps there were remote common founders.)

Zhang ZP, Blomback M, Nyman D, Anvret M. Mutations of von Willebrand factor gene in families with von Willebrand disease in the Åland islands. *Proc Natl Acad Sci* 1993; 90:7937-7940.

A cytosine deletion causing a stop codon was the causative VWF gene mutation in four families with VWD living in the Åland islands, including members of the family described by von Willebrand. (Three of the families were known to be related as of church records of the 1650's.) 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 suggests that many Swedish patients were related to the Åland island patients. (The survival of this mutation over centuries is noteworthy, perhaps due to mild phenotypic expression in heterozygotes.)

Zhang ZP, Blomback M, Egberg N, Falk G, Anvret M. Characterization of the von Willebrand factor gene (VWF) in von Willebrand disease type III patients from 24 families of Swedish and Finnish origin. *Genomics* 1994; 21:188-193.

Mutations were found in 42/48 VWF genes in 24 patients with type 3 VWD. Eleven patients were homozygotes, eight were compound heterozygotes, and the remaining five patients were heterozygotes.

Schneppenheim R, Krey S, Bergmann F, Bock D, Budde U, Lange M, Linde R, Mittler U, Meili E, Mertes G et alia. Genetic heterogeneity of severe von Willebrand disease type III in the German population. *Hum Genet* 1994; 94:640-653.

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 were correlated with asymptomatic heterozygotes, whereas frameshift and nonsense mutations in the sequence corresponding to the mature subunit tended to result in a type 1 VWD phenotype in heterozygotes.

Eikenboom JCJ, Castaman G, Vos HL, Bertina RM, Rodeghiero F. Characterization of the genetic defects in recessive type 1 and type 3 von Willebrand disease patients of Italian origin. *Thromb Haemost* 1998; 79: 709-711.

Eight heterozygous relatives of patients with severe VWD had mildly subnormal levels of VWF, which might lead to a diagnosis of type 1 VWD, although only two had (mild) bleeding symptoms. Fourteen other heterozygotes had normal phenotypes.

Zhang Z, Lindstedt M, Blomback M, Anvret M. Effects of the mutant von Willebrand factor gene in von Willebrand disease. *Hum Genet* 1995; 96:388-394.

A man with an almost-normal phenotype had a mutation in each of his two VWF genes. One was inherited by a daughter with type 3 VWD and the other was inherited by three other 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. Three different mutations were found in these families, one of which, Arg1853X, was associated with lower levels of VWF:Ag than were the other two mutations, as shown in the accompanying table. There was notable variation in clinical symptoms and VWF levels in persons with the same mutation, as follows:

Mutation on one gene	Subjects, N	VWF:Ag, %, mean	Median, %	Range, %
2680delC	37	46	43	13-110
Arg1659X	11	47	42	12-94
Arg1853X	7	22	19	13-39

Baronciani L, Cozzi G, Canciani MT, Peyvandi F, Srivastava A, Federici AB, Mannucci PM. Molecular characterization of a multiethnic group of 21 patients with type 3 von Willebrand disease. *Thromb Haemost* 2000; 84:536-540.

Genotypes were determined in six unrelated patients from Italy, nine from Iran and six from India with type 3 VWD. Nine were known to come from consanguineous unions. Eighteen patients were homozygotes (with 18 different mutations) and three were compound heterozygotes (with different mutations on each of the six genes.). Mutations were scattered throughout the gene. The majority were null alleles: including five deletions, two insertions causing premature stop codons, 11 nonsense mutations, and three possible splice site mutations. Three missense mutations were found, two as homozygous mutations.

Baronciani L, Cozzi G, Canciani MT, Peyvandi F, Srivastava A, Federici AB, Mannucci PM. Molecular defects in type 3 von Willebrand disease: Updated results from 40 multiethnic patients. *Blood Cells Mol Dis* 2003; 30: 264-270.

The study of 2000 (above) was updated, with a total of 40 patients with type 3 VWD. Fifty 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.

Castaman G, Rodeghiero F, Tosoletto A, Cappelletti A, Baudo F, Eikenboom JC, Federici AB, Lethagen S, Linari S, Lusher J, Nishino M, Petrini P, Srivastava A, Ungerstedt JS. Hemorrhagic symptoms and bleeding risk in obligatory carriers of type 3 von Willebrand disease: an international multicenter study. *J Thromb Haemost* 2006; 4:2164-2169.

At least one bleeding symptom was reported by about 40% of 70 obligatory carriers (parents and children) of patients with type 3 VWD, compared to 82% of 42 obligatory carriers of type 1 VWD. Carriers of type 3 VWD have more frequent bleeding problems than do normal persons.

### Pseudo-VWD (Platelet-type VWD)

Weiss HJ, Meyer D, Rabinowitz R, Pietu G, Girma JP, Vivic WJ, Rogers J: Pseudo-von Willebrand's disease: An intrinsic platelet defect with aggregation by unmodified human factor VIII/von Willebrand factor and enhanced adsorption of its high molecular weight multimers. *N Engl J Med* 1982; 306:326-333

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.

Miller JL, Castella A: Platelet-type von Willebrand's disease: Characterization of a new bleeding disorder. *Blood* 1982; 60:790-794.

Five patients in three generations of a family with a dominant form of VWD had slightly prolonged BTs, normal FVIII (64-97%) and VWF:Ag (52-200%), decreased VWF:RCo (<12.5-30%), and absent HMW multimers. They also had low-normal platelet counts (140,000 – 170,000/cu mm) and enhanced RIPA. Binding of patient VWF to washed normal platelets was normal, whereas binding of normal VWF to patient platelets was significantly increased. The authors presumed this to be an intrinsic platelet abnormality and called it "platelet-type von Willebrand's disease".

Miller JL, Kupinski JM, Castella A, Ruggeri ZM. Von Willebrand factor binds to platelets and induces aggregation in platelet-type but not type IIB von Willebrand disease. *J Clin Invest* 1983; 72:1532-1542.

**VWF induced aggregation in platelets from patients with pseudo-VWD was normal but it was abnormal in platelets from patients with type 2B VWD, distinguishing the two disorders. The authors suggest that the low levels of plasma VWF:RCo in these patients was due to prior *in vivo* platelet-VWF interaction with removal of HMW multimers.**

Takahashi H, Okada K, Abe S, Wada K, Nagayama R, Tawaki W, Hanano M, Takizawa S, Shibata A. Platelet aggregation induced by cryoprecipitate infusion in platelet-type von Willebrand's disease. *Thromb Haemost* 1987; 46:255-262.

**Cryoprecipitate given to a patient with this disorder caused thrombocytopenia *in vivo* and "spontaneous" platelet aggregation *in vitro*. A prolonged BT was shortened and hemostasis was adequate.**

Casonato A, De Marco L, Mazzucato M, De Angelis V, De Roia D, Fabris F, Ruggeri ZM, Girolami A. A new congenital platelet abnormality characterized by spontaneous platelet aggregation, enhanced von Willebrand factor platelet interaction, and the presence of all von Willebrand factor multimers in plasma. *Blood* 1989; 74:2028-2033

**A woman with a mild bleeding tendency had a BT, VWF:RCo, VWF:Ag and multimers within normal limits. Her platelet count was normal but platelets in her platelet-rich plasma aggregated spontaneously *in vitro* and with low concentrations of ristocetin. Platelet aggregation was initiated by purified VWF alone. (This patient's disorder might not have been discovered had the doctors not persevered to perform RIPA despite finding other test results within normal limits.)**

Russell SD, Roth GJ. Pseudo-von Willebrand disease: A mutation in the platelet glycoprotein Iba gene associated with a hyperactive surface receptor. *Blood* 1993; 81:1787-1791.

**A point mutation was found in the platelet GPIb gene from persons with pseudo-VWD but not from normal persons. Affected persons had a mutant and a normal gene, i.e., were heterozygotes.**

Scott JP, Montgomery RR. The rapid differentiation of type IIB von Willebrand's disease from platelet-type (pseudo-) von Willebrand's disease by the "neutral" monoclonal antibody binding assay. *Am J Clin Path* 1993; 96: 723-728.

**Laboratory differentiation of type 2B and pseudo-VWD is difficult. A monoclonal non-neutralizing antibody to VWF was used as a label for VWF. The binding of VWF to formalin-fixed washed normal platelets was studied as a function of ristocetin concentration. Labeled VWF from type 2B VWD bound at a significantly lower concentration of ristocetin than did labeled VWF from normal persons or persons with pseudo-VWD. (The assay described is suitable for a reference laboratory.)**

Miller JL. Platelet-type von Willebrand disease. *Thromb Haemost* 1996; 75:865-869. (Review, 42 refs.)

Enayat MS, Guilliatt AM, Lester W, Wilde JT, Williams MD, Hill FGH. Distinguishing between type 2B and pseudo-von Willebrand disease and its clinical importance. *Br J Haematol* 2006; 133:664-666.

**In 10 patients from five families with the G233V mutation in the GP1BA gene, RIPA was increased and platelets aggregated upon addition of cryoprecipitate. The authors believed that the latter test was a good determinant of the families' pseudo-VWD as opposed to type 2B VWD, and that pseudo-VWD may be under-diagnosed.**

Favaloro EJ. 2B or not 2B? Differential identification of type 2B, versus pseudo-von Willebrand disease. *Br J Haematol* 2006; 135:141-142

**Commenting on the above paper, this very experienced laboratory diagnostician comments that platelets from patients with type 2B VWD may also aggregate on the addition of cryoprecipitate.**

Whalley IN, Perry DJ. 2B or not 2B? Differential identification of type 2B, versus pseudo-von Willebrand disease. *Br J Haematol* 2006; 136:345.

**Commenting on the above, A kindred with 8 affected members is long thought to have type 2B were found to have no mutation in the VWF gene but had a G249V mutation in the GP1BA gene.**

Othman M, Lillicrap D. Distinguishing between non-identical twins: platelet type and type 2B von Willebrand disease. *Br J Haematol* 2007; 138:665-666.

**In response to the above, Among 14 patients presumed to have type 2B referred for genetic testing in Canada, eight had mutations in exon 28 of the VWF gene and two had a G233V mutation in the GP1BA gene. Genotyping in a central laboratory is less prone to error than RIPA mixing studies. Funding has been received for an international trial to clarify the relative frequency of the two conditions.**

### Acquired von Willebrand disease

Simone JV, Cornet JA, Abildgaard CF. Acquired von Willebrand's syndrome in systemic lupus erythematosus. *Blood* 1968; 31:806-812.

**A 12-year-old boy with prior normal hemostasis, including surgery, began to have epistaxis and bruising, and bled excessively after a dental extraction. A diagnosis of VWD was made on the basis of a prolonged BT, reduced platelet adhesiveness, and a FVIII level of 17%. Two years later symptoms of lupus erythematosus appeared. The acquired VWD and the SLE responded simultaneously to corticosteroids. (Acquired VWD may appear before symptoms of an underlying disorder.)**

Ingram GIC, Kingston PJ, Leslie J, Bowie EJW. Four cases of acquired von Willebrand's syndrome. *Br J Haematol* 1971; 21:189-199.

**In four patients, a bleeding disorder developing *de novo* in adult life was characterized by prolonged BTs, reduced levels of FVIII and excessive bleeding from skin and mucosal injuries. One patient who also had systemic lupus erythematosus responded to corticosteroids.**

Handin RI, Martin V, Moloney WC. Antibody-induced von Willebrand's disease: A newly defined inhibitor syndrome. *Blood* 1976; 48:393-405.

**A patient with lymphosarcoma had a prolonged BT and marked reductions in FVIII, VWF:Ag and VWF:RCo. His plasma contained an inhibitor which prevented aggregation of normal platelets by ristocetin but did not interfere with measurement of VWF:Ag or FVIII.**

Mannucci PM, Lombardi R, Bader R, Horellou MH, Finazzi G, Besana C, Conard J, Samama M. Studies of the pathophysiology of acquired von Willebrand's disease in seven patients with lymphoproliferative disorders or benign monoclonal gammopathy. *Blood* 1984; 64:614-621.

**In seven patients with acquired VWD, platelet content of VWF was normal with multimers of all sizes. Plasma VWF:Ag was decreased but multimers of all sizes were present. DDAVP infusion raised plasma levels of VWF but to a lesser extent, and with a shorter half-life, than in congenital VWD. (A plasma test for a VWF inhibitor is described in detail.)**

Dalton RG, Dewar MS, Savidge GF, Kernoff PB, Matthews KB, Greaves M, Preston FE. Hypothyroidism as a cause of acquired von Willebrand's disease. *Lancet* 1987; 1: 1007-1009.

**In two patients, hypothyroidism was suspected at the time of diagnosis of acquired VWF and in a third, hypothyroidism became apparent four years later. Both conditions improved with thyroxine therapy.**

Uehlinger J, Button GR, McCarthy J, Forster A, Watt R, Aledort LM. Immunoabsorption for coagulation factor inhibitors. *Transfusion* 1991; 31:265-269.

**Plasmapheresis with immunoabsorption was used to try to reduce antibody levels in various patients with clotting factor inhibitors, including one with acquired VWD. The procedure apparently reduced the (unmeasurable) antibody on one occasion, and subsequent replacement**

therapy was successful.

Warkentin TE, Moore JC, Morgan DG. Aortic stenosis and bleeding gastrointestinal angiodysplasia: is acquired von Willebrand's disease the link? *Lancet* 1992; 340: 35-37.

When aortic stenosis and bleeding from gastrointestinal angiodysplasia co-existed, the bleeding resolved with replacement of the aortic valve. Aortic stenosis can be complicated by acquired VWD resembling type 2A because HMW multimers are absent. High shear stress at the abnormal valve may consume HMW multimers, causing acquired type 2A VWD and then increased bleeding from angiodysplasia.

Vincentelli A, Susen S, Le Tourneau T, Six I, Fabre O, Juthier F, Bauters A, Decoene C, Goudemand J, Prat A, Jude B. Acquired von Willebrand syndrome in aortic stenosis. *N Engl J Med* 2003; 349:343-349.

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% with moderate stenosis. HMW multimers were low in 79% of patients. In 42 patients having surgical correction with prostheses or biological implants, the platelet function analyzer CT had become normal, as was the multimer distribution, on post-op days one and seven. In some patients, in particular those with a mismatch of the prosthesis or with homograft stenosis, a loss of HMW multimers recurred, again prolonging CTs.

Soff GA, Green D. Autoantibody to von Willebrand factor in systemic lupus erythematosus. *J Lab Clin Med* 1993; 121:424-430.

After mixing experiments failed to prove the presence of an inhibitor to VWF in a patient, a more sensitive ELISA test was devised. The ELISA measured the binding of anti-VWF antibody from plasma to surface-bound VWF:Ag. Binding was detected by a conjugate of anti-human-immunoglobulin with alkaline phosphatase and a chromogenic substrate for the phosphatase. The test was validated with the plasma of a patient with type 3 VWD and a known antibody to VWF.

Staka C, Rugeri L, Caron C, Goudemand J. A new ELISA assay for diagnosis of acquired von Willebrand syndrome. *Haemophilia* 2003; 9:303-308.

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. Among the ten patients screened, six had a monoclonal gammopathy, two had Waldenstrom's macroglobulinemia, one had scleroderma, and one had no known underlying disorder.

Van Genderen PJJ, Boertjes RC, van Mourik JA. Quantitative analysis of von Willebrand factor and its propeptide in plasma in acquired von Willebrand syndrome. *Thromb Haemost* 1998; 80:494-498.

Plasma levels of mature VWF:Ag were significantly lower in eight persons with acquired VWD than in 20 normal persons whereas levels of the propeptide were significantly higher (11.4 nM, SD 1.1) than normal (4.7nM, SD 0.2). Measurement of propeptide levels distinguish congenital from acquired VWD. (A potential reference laboratory test.)

Nitu-Whalley IC, Lee CA. Acquired von Willebrand syndrome - report of 10 cases and review of the literature. *Haemophilia* 1999; 5:328-326. (with a brief review and 67 references)

Ten adults with acquired VWD were seen at the Royal Free Hospital, London over 17 years. Presenting symptoms were post-operative bleeding in five patients, excessive bruising in three, epistaxis in three, gastrointestinal bleeding in one, menorrhagia in one (three had more than one symptom.) Underlying disorders included hypothyroidism in the two youngest patients ages 22 and 34 years, multiple myeloma in three, lymphoma in one, macroglobulinemia in one, monoclonal gammopathy with carcinoma and autoimmune disorders in one, and undetermined in two. In contrast to other reports, a normal multimer pattern was seen in a majority (8 patients). Attempts to demonstrate neutralizing anti-

bodies were successful in only two patients. Treatment for bleeding with DDAVP or clotting factor concentrates resulted in brief elevations of clotting factor levels that were adequate for hemostasis.

Vayradier A, Jenkins CSP, Fressinaud E, Meyer D. Acquired von Willebrand syndrome: From pathophysiology to management. *Thromb Haemost* 2000; 84:175-82. Review, 133 references.

Clinical symptoms of acquired VWD were similar to those of congenital VWD. Common concurrent diseases include clonal hematoproliferative, neoplastic and autoimmune disorders. VWF was synthesized and secreted normally. Mechanisms for its low concentration in plasma are as follows: (1) Circulating antibodies may be present. Specific autoantibodies have been described that interfere with VWF binding to platelet GP1b, or that interfere with binding to collagen, or that interfere with binding to GPIIb/IIIa. (2) VWF, especially the HMW multimers, may be selectively adsorbed from plasma onto malignant cells or activated platelets. (3) Increased proteolysis of VWF may be induced, as by the action of various enzymes such as the protease and elastase secreted by cells associated with myeloproliferative disorders, or by increased plasmin. (4) HMW multimers may be adsorbed by activated platelets in areas of chronic high shear-stress such as aortic stenosis.

The few tests suggesting that VWF is acquired (see above papers for tests) are mainly available only in a research laboratory.

Treatment included a search for an underlying disorder and its appropriate treatment. Attempts to control bleeding with infusions of DDAVP or with concentrates containing VWF were sometimes briefly helpful. Infusions of intravenous gamma-globulin were helpful in patients lymphoproliferative disorders or monoclonal gammopathies.

Federici AB, Rand JH, Bucciarelli P, Budder U, van Genderen PJJ, Mohri H, Meyer D, Rodeghiero F, Sadler JE. Acquired von Willebrand syndrome: Data from an international registry. *Thromb Haemost* 2000; 84: 345-349

The literature up to 1999 contained 266 published cases of acquired VWD. An international survey was undertaken and information gathered on 186 additional patients to form this report. Underlying disorders included lymphoproliferative (48%) and myeloproliferative (15%) disorders, neoplasia (5%), immunological disorders (1%), cardiovascular disorders (21%) and others. 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, whereas 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 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 episodes were mostly of the muco-cutaneous type. 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.

Kumar S, Pruthi RK, Nichols WL. Acquired von Willebrand's disease. *Mayo Clin Proc* 2002; 77:181-187. Review, 101 references, or, similar, Kumar S, Pruthi RK, Nichols WL. Acquired von Willebrand's syndrome: a single institution experience. *Am J Hematol* 2003; 72:243-247.

At the Mayo Clinic, 22 patients with acquired VWD were seen over 25 years. The mean age at diagnosis was 61.3 years. Gastrointestinal bleeding and epistaxis were the most common presenting symptoms. A variety of multimer patterns were seen including type 1 patterns with all multimer sizes present. No inhibition of VWF:RCo was found on mixing studies in 17 tested patients. An underlying hematologic disease was found in 18 patients. DDAVP was effective in half the patients so treated, but FVIII-VWF was effective in all. The authors suggested that acquired VWD may be more prevalent than previously appreciated.

Federici AB. Use of intravenous immunoglobulin in patients with acquired von Willebrand syndrome. *Hum Immunol* 2005; 66:422-430.

**Intravenous immunoglobulin is most effective in patients with IgG autoantibodies, especially with monoclonal gammopathies.**

### VWD and vascular lesions

*From time to time, ever since the original report of Dr. von Willebrand, the question of a concomitant vascular lesion has been raised.*

Macfarlane RG. Critical Review: The mechanisms of haemostasis. *Quart J Med* 1941; 10:1-32.

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 was hemorrhage lasting a few seconds, then disappearance of the vessel, presumably because it had been emptied of visible red cells. Dr. Macfarlane believed that the BT is a measurement of the reactivity of the capillaries. He studied five women with bruising and prolonged BTs but normal platelet counts, whom he believed had the condition described by von Willebrand. "The capillaries ... were of distorted and often bizarre forms, and did not contact after injury..." He differentiates this from telangiectasia: "in which there is a sharply localized abnormality of the capillaries." *The respected author's excellent pictures show tortuosity in VWD but dilated capillary loops in telangiectasia.*

Levy II L. Non-hemophilic. Non-hemophilic hereditary hemorrhagic diathesis: Report of a family of bleeders. *Ann Intern Med* 1947; 27:96-102.

Twenty persons in a family had a congenital bleeding disorder. Epistaxis and other mucosal bleeding were prominent. In five affected persons, platelet counts, capillary fragility tests and clotting times were performed and were normal but, in four of the patients, the Duke BT was prolonged. "Microscopic studies of the capillaries in the nailbeds...in three patients...revealed tortuosity of the capillary loops and failure of the blood cell columns to disappear after injury..."

Alexander B, Goldstein R. Dual hemostatic defect in pseudohemophilia. *J Clin Invest* 1953; 32:581.

Two unrelated patients with bleeding disorders are described. The FVIII level was 5-10% in a severely-affected child whose affected sibling had died of bleeding, and 10-20% in a mildly-affected unrelated adult. Both had "irregular and distorted" capillaries in the nail bed.

Quick AJ. Telangiectasia: its relationship to the Minot-von Willebrand syndrome. *Am J Med Sci* 1967; 254:585-601.

In hereditary hemorrhagic telangiectasia, epistaxis is common in youth, cutaneous lesions become apparent in the second or third decade and gastro-intestinal bleeding emerges in middle age or later. A few published reports had suggested co-existence of VWD and telangiectasia in some patients. Quick commented that the diagnosis of telangiectasia, like VWD, may be elusive. Physicians may overlook a small solitary external telangiectatic lesion, if not alert to the possibility. Lesions increase in frequency with age. A causal association with VWD is not proven.

Ahr DJ, Rickles FR, Hoyer LW, O'Leary DS, Conrad ME: Von Willebrand's disease and hemorrhagic telangiectasia: Association of two complex disorders of hemostasis resulting in life-threatening hemorrhage. *Am J Med* 1977; 62:452-458.

A woman with familial VWD, with FVIII 13% and VWF:Ag 4%, had frequent gastrointestinal hemorrhages from age 48 until her death at age 62. Telangiectatic lesions were found in the stomach, duodenum and sigmoid colon on endoscopy. On laparotomy, telangiectases were found to be scattered throughout the colon. She had no external telangiectasia, nor could any be found in her relatives with VWD. The authors wondered whether the concurrence of telangiectasia and VWD is rare.

Conlon CL, Weinger RS, Cimo PL, Moake JL, Olson JD. Telangiectasia and von Willebrand's disease in two families. *Ann Intern Med* 1978; 89:921-924.

In one family with with four VWD-affected members in three generations, all had telangiectasia. In another family with 12 living VWD-

affected members in four generations, two also had telangiectasia.

Hanna W, McCarroll D, Lin D, Chua W, McDonald TP, Chen J, Congdon C, Lange RD. A study of a Caucasian family with variant von Willebrand's disease in association with vascular telangiectasia and haemoglobinopathy. *Thromb Haemost* 1984; 51:276-278.

**Fifteen persons in four generations of a family had type 2A VWD. Six had telangiectasia in mucous membranes and skin; two who had examinations of the stomach also had telangiectasia in that location.**

Fressinaud E, Meyer D. International survey of patients with von Willebrand's disease and anigodysplasia (letter). *Thromb Haemost* 1993; 70:546.

It is not clear whether angiodysplasia in VWD is a coincidence or whether there is a causal relationship. Angiodysplasia may be a degenerative process of aging. An international survey covered 4,503 patients with VWD, 1.2% with acquired VWD. The prevalence of angiodysplasia in acquired VWD was 11.7%; median age at diagnosis 69 years. In congenital VWD, angiodysplasia was found exclusively in patients with type 2 VWD (2%) or type 3 VWD (4.5%) at a median age of 55. Perhaps angiodysplasia is not more frequent in VWD but, if present, is more likely to be associated with hemorrhage, especially if large multimers are absent.

Koscielny JK, Latza R, Mursdorf S, Mrowietz C, Kiesewetter H, Wenzel E, Jung F. Capillary microscopic and rheological dimensions for the diagnosis of von Willebrand disease in comparison to other haemorrhagic diatheses. *Thromb Haemost* 2000; 84:981-988. (*Good pictures*)

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), 122 with thrombocytopenia, 101 with thrombocytopenia, 50 with severe hemophilia A, 20 with severe hemophilia B, 22 with congenital dysfibrinogenemia and 112 persons on coumadin therapy. Only the VWD subjects had significantly increased tortuosity of the capillaries, dilation of venules and arterioles, and extravasates from capillaries. Only in dysfibrinogenemia are venules dilated. The authors commented that the phenomenon had been neglected in recent decades.

Morris ES, Hampton KK, Nesbitt JM, Preston FE, Thomas WEG, Makris M. The management of von Willebrand's disease—associated gastrointestinal angiodysplasia. *Blood Coag Fibrinol* 2001; 12:143-148.

Four patients with type 2A and two with acquired VWD had gastrointestinal bleeding. Angiodysplasia was demonstrated in 5 patients and presumed in one (who had acquired VWD). Estrogen-progesterone treatment was effective in two patients able to tolerate the hormones. The authors pointed out 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.

Veyradier A, Balian A, Wolf M, Giraud V, Montebault S, Obert B, Dagher I, Chaput JC, Meyer D, Naveau S. Abnormal von Willebrand factor in bleeding angiodysplasias of the digestive tract. *Gastroenterology* 2001; 120:346-353.

VWF in nine patients with non-bleeding angiodysplasia or telangiectasia of the GI tract was compared to that of nine patients with bleeding lesions. Large multimers were lost in eight of nine patients with bleeding lesions. In seven patients, VWD probably was acquired secondary to aortic stenosis.

### VWD and atherosclerosis

*The possibility that a bleeding disorder such as VWD might protect the patient against atherosclerotic plaques was addressed by these investigators..*

Silwer J, Cronberg S, Nilsson IM. Occurrence of arteriosclerosis in von Willebrand's disease. *Acta Med Scand* 1966; 180: 474-484.

In autopsies were performed on three patients with VWD (moderately severe by symptoms) over age 40, early atherosclerosis was found in a 54-year-old, and advanced atherosclerosis in patients aged 69 and 72 years, respectively. Symptoms and signs of atherosclerosis were found in 14 of 31 living VWD patients over 40 years old.

Fuster V, Bowie EJW, Lewis JC, Fass DN, Owen CA, Brown AL. Resistance to



arteriosclerosis in pigs with von Willebrand's disease. *J Clin Invest* 1978; 61:722-730.

The aortas of 11 pigs with homozygous 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 < 2 mm and 11 were larger. Four VWD pigs had one lesion each, but only one of these lesions > 2 mm. In another experiment, 11 normal and seven 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 three had fewer than seen in the control pigs.

Badimon L, Steele P, Badmino JJ, Bowie EJW, Fuster V. Aortic atherosclerosis in pigs with heterozygous von Willebrand disease: comparison with homozygous von Willebrand and normal pigs. *Arteriosclerosis* 1985; 5:366-370.

Pigs were fed a high fat, high cholesterol diet from age 3 to 9 months. Nine normal pigs had a mean of 21% atherosclerotic involvement of the distal aorta. Five pigs homozygous for type 3 VWD had a mean of only 4.2% atherosclerosis in that area. In contrast, five heterozygous pigs had 25% involvement, i.e., not different from normal.

Federici AB, Mannucci PM, Fogato E, Ghidoni P, Maturri L. Autopsy findings in three patients with von Willebrand disease type IIB and type III: Presence of atherosclerotic lesions without occlusive arterial thrombi. *Thromb Haemost* 1993; 70:758-761.

Autopsies were conducted on a 73 year old patient with type 2B VWD and two patients, ages 44 and 52 respectively, with type 3 VWD. Atherosclerosis was found in all patients but were fewer and smaller in the type 3 patients than in the type 2B patient.

Sramek A, Bucciarelli P, Federici AB, Mannucci PM, De Rosa V, Castaman G, Morfini M, Mazzucconi MG, Rocino A, Schiavoni M, Rocino A, Schiavoni M, Scaraggi FA, Reiber JH, Rosendaal FR. Patients with type 3 severe von Willebrand disease are not protected against atherosclerosis: results from a multi-center study in 47 patients. *Circulation* 2004; 109:740-744.

Ultrasonography measured intimal thickening and atherosclerotic plaques in the carotid and femoral arteries of 47 patients with type 3 VWD and 84 controls. No difference between the groups was found.

## Symptoms

Foster, PA. The reproductive health of women with von Willebrand disease unresponsive to DDAVP: Results of an international survey. *Thromb Haemost* 1995; 74:784-790. (Review)

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

Kouides PA, Phatek PD, Burkart P, Braggins C, Cox C, Bernstein Z, Belling L, Holmberg P, MacLaughlin W, Howard F. Gynecological and obstetrical morbidity in women with type I von Willbrand disease: results of a patient survey. *Haemophilia* 2000; 6:643-648.

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. Higher numbers of women with VWD reported menorrhagia, anemia and hemorrhaging at delivery necessitating red blood cell transfusion, compared to normal women, at a high level of significance. 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 control women.

Ziv O, Ragni M. Bleeding manifestations in males with von Willebrand disease. *Haemophilia* 2004; 10:162-168.

In a retrospective review of 47 males (>90% under age 21) diagnosed with type 1 VWD, the most common bleeding symptoms were as follows: epistaxis in 52.6%; easy bruising 50%; post-operative 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%; hemarthroses 7.9%; gastrointestinal bleeding 7.9% and hematuria 5.3 %.

Kirtava A, Crudder S, Dilley A, Lally C, Evatt B. Trends in clinical management of women with von Willebrand disease: a survey of 75 women enrolled in haemophilia treatment centres in the United States. *Haemophilia* 2004; 10:128-61.

Among 75 adult women with type 1 VWD, mean age 40, at haemophilia 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.

## Measurement of Menstrual Blood Loss

*Menorrhagia is a frequent complaint in general and a particular problem in VWD, but it has been difficult to quantitate until recent years.*

Hallberg L, Hogdahl AM, Nilsson L, Rybo G. Menstrual blood loss – a population study. Variation at different ages and attempts to define normality. *Acta Obstet Gynecol Scand* 1966; 45:320-251

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, median 29.9 ml. In 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, > 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% considered it heavy.

Higham JM, O'Brien PMS, Shaw RW. Assessment of menstrual blood loss using a pictorial chart. *Br J Obstet Gynaecol* 1990; 97:734-9.

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.

Janssen CAH, Scholten PC, Heintz APM. A simple visual assessment technique to discriminate between menorrhagia and normal menstrual blood loss. *Obstet Gynecol* 1995; 85:977-982.

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 (above). Two consecutive menstrual cycles were tested in 201 women; the degree of bleeding in the second cycle was similar to the first. The PBAC had a good level of reliability and scoring just one cycle sufficed.

## Menorrhagia in VWD

Kadir R, Economides DL, Sabin CA, Owens D, Lee CA. Frequency of inherited bleeding disorders in women with menorrhagia. *Lancet* 1998; 351:485-489.

Among 150 women referred to hematologists because of menorrhagia, with an estimated menstrual blood loss of more than 80 ml, 26 proved to have some inherited bleeding abnormality, and 18 of those had VWD. VWD was diagnosed if VWF:Ag was less than 50% in two of three blood samples taken before day 7 of the menstrual cycle. Of the 18, three had moderate and 15 had mild VWD; 11 had blood group O.

Kadir RA, Economides DL, Sabin CA, Pollard D, Lee CA. Assessment of menstrual blood loss and gynaecological problems inpatients with inherited bleeding disorders. *Haemophilia* 1999; 5:40-48.

A PBAC score of > 100, confirming menorrhagia, was found in 74% of women with VWD. (All types of VWD were considered together,

but 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 but the difference was not statistically significant.

Ragni MV, Bontempo RA, Hassett AC. Von Willebrand disease and bleeding in women. *Haemophilia* 1999; 5: 313-317.

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

Lee CA. Women and von Willebrand disease. *Haemophilia* 1999; 5 (suppl 2), 38-45. (a brief review)

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

Dilley A, Drews C, Miller C, Lally C, Austin H, Ramaswamy D, Lurye D, Evatt B. Von Willebrand disease and other inherited bleeding disorders in women with diagnosed menorrhagia. *Obstet Gynecol* 2001; 97:630-636.

The authors examined 121 women with menorrhagia, from the records of one insurance agency in the southeast USA, and 123 normal women. Normal ranges for laboratory tests were established according to ABO blood group and race (black vs. white). VWD was diagnosed in seven (15.9%) white women with menorrhagia but only one (1.4%) black woman with menorrhagia and in one (1.3%) black control woman.

Bevan JA, Maloney KW, Hillery CA, Gill JC, Montgomery RR, Scott JP. Bleeding disorders: A common cause of menorrhagia in adolescents. *J Pediatr* 2001; 138: 856-861.

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

Saxena R, Gupta M, Gupta PK, Kashyap R, Choudhry VP, Bhargava M. Inherited bleeding disorders in Indian women with menorrhagia. *Haemophilia* 2003; 9:193-196.

Over a nine month period, 2200 women were referred from the Department of Gynecology to that of Haematology at the All India Institute of Medical Sciences (New Delhi). Inherited bleeding disorders were found in 337 (15.3%). The most frequent were platelet disorders (283 cases). Von Willebrand disease, in 40 women, was the most common plasma clotting factor disorder. (About two cases of VWD per 100 symptomatic women).

Kujovich JL. Von Willebrand's disease and menorrhagia: prevalence, diagnosis and management. *Am J Hematol* 2005; 79:220-228. Review.

Lee CA, Abdul-Kadir R. Von Willebrand disease and women's health. *Semin Hematol* 2005; 42: 42-28. Review

### Pregnancy in VWD

Strauss HS, Diamond LK. Elevation of factor VIII (antihemophilic factor) during pregnancy in normal persons and in a patient with von Willebrand's disease. *New Engl J Med* 1963; 269: 1251-1252.

Factor VIII levels were followed in seven normal women during pregnancy. At term, levels ranged from 140 to 480%. A woman with VWD with a very prolonged BT and a baseline FVIII level of 12% reached a FVIII level of 47% with a BT improved almost to normal at term. The woman had excessive bleeding with surgical procedures but not with her three term deliveries. (A history of no excess bleeding at deliveries can give the unwary a false sense of security about surgical operations.)

Winckelmann G, Groh R, Schneider J, Huber P. Pregnancy and child-birth in von Willebrand's disease. *Germ. Med. Mth.* 1967; 12:208-211.

FVIII levels at term in 25 normal pregnant women averaged 246% at term. Levels fell by the third day after delivery. A woman with severe VWD with a prolonged BT and a FVIII level of <1% did not improve at term; her delivery was followed by heavy hemorrhaging.

Lipton R, Ayromloo J, Collier BS. Severe von Willebrand's disease during labor and delivery. *JAMA* 1982; 248:1355-1357.

A woman with VWD had 24% FVIII, 3% VWF:Ag, 10% VWF:RCo and a very prolonged BT before pregnancy. At term, her FVIII level rose to 50% but there was no rise of VWF:Ag or of VWF:RCo. Abundant cryoprecipitate was transfused beginning in the second stage of labor continuing until four days after delivery. At eight days, however, bleeding recurred and further cryoprecipitate was required.

Another woman with VWD had 22.5% FVIII, 31.5% VWF:Ag, 19% VWF:RCo and a very prolonged BT before pregnancy. During pregnancy, levels of FVIII, VWF:Ag and VWF:RCo all rose well into the normal range. Cryoprecipitate was given before and for two days after a caesarean section; no excessive bleeding ensued. *This reference contains excellent graphs of the levels of FVIII and VWF during term and in the puerperium.*

Chediak JR, Alban GM, Maxey B. Von Willebrand's disease and pregnancy: Management during delivery and outcome of offspring. *Am J Obstet Gynecol* 1986, 155: 618-624.

Eight pregnancies in six patients are described. In three women with type 1 VWD, the BT, FVIII and VWF markedly improved by term. In two women with type 2A and one with type 3 VWD, levels of FVIII rose by term but VWF rose little or not at all. The woman with type 3 VWD bled excessively with each of two deliveries, requiring factor replacement. One woman with type 2A VWD also had bleeding with each of two deliveries requiring factor replacement. The other woman with type 2A had an elective cesarean section after being prepared with cryoprecipitate and had no hemorrhage. Two of three women with type 1 VWD were given cryoprecipitate for delivery; none of the three had excessive bleeding.

Greer IA, Lowe GDO, Walker JJ, Forbes CD. Haemorrhagic problems in obstetrics and gynaecology in patients with congenital coagulopathies. *Br J Obstet Gynaecol* 1991; 98:909-918.

Over a 30 year period in Glasgow, there were 14 pregnancies among eight women with VWD (three with type 1 VWD, five with type 2). All had prolonged BTs and definitely reduced VWF:RCo. No cryoprecipitate was given at five of the deliveries; in two of these (both in a woman with type 2A VWD) early postpartum hemorrhage occurred. Cryoprecipitate was given at nine other deliveries and in five of these deliveries (all in women with type 2 VWD) marked hemorrhage ensued despite the precautionary cryoprecipitate.

Ramsahoye BH, Davies SV, Dasani H, Pearson JF. Obstetric management in von Willebrand's disease: a report of 24 pregnancies and a review of the literature. *Haemophilia* 1995; 1:140-144.

Thirteen patients, seven with type 1 VWD and six with 2A or 2B, had 24 pregnancies. 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). Hemorrhage was more likely in type 2 patients especially if no prophylaxis had been given.

VWD type	Management at delivery	Primary hemorrhage	Secondary hemorrhage
Type 1	Prophylaxis	0/5	0/5
Type 1	No prophylaxis	0/8	3/8
Type 2	Prophylaxis	0/5	2/5
Type 2	No prophylaxis	3/6	1/6

Caliez C, Tsakiris Da, Behringer H, Kuhne T, Marbert GA. Two consecutive pregnancies and deliveries in a patient with von Willebrand's disease type 3. *Haemophilia* 1998; 4:845-849.

A woman with severe VWD had two pregnancies. Prophylactic concentrate was given at the onset of labor, at delivery and daily for seven days afterwards but she re-bled on post-delivery days 15 and 22 and was given further concentrate. Concentrate was again given for the second delivery for labor, delivery and the early puerperium and as prophylaxis for four weeks after delivery, with no further bleeding. Episiotomies were performed in both deliveries. Spinal anesthesia was given for the first delivery. Both pregnancies were attended by increased epistaxis.

Kadir RA, Lee CA, Sabin CA, Pollard D, Economides D. Pregnancy in women with von Willebrand's disease or factor XI deficiency. *Br J Obstet Gynecol* 1998; 105:314-321.

Over 17 years, 84 pregnancies in 31 women with VWD (27 type 1, two type 2, two type 3) were seen. 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, necessitating blood transfusion in six instances. Secondary hemorrhage was reported in eleven of 54 deliveries, with three instances of blood transfusion. No postpartum hemorrhage occurred in the ten women with VWD who had been given prophylactic treatment at the time of delivery. (*The VWD type in cases of postpartum hemorrhage was not identified.*)

Lak M, Peyvandi F, Mannucci PM. Clinical manifestations and complications of childbirth and replacement therapy in 385 Iranian patients with type 3 von Willebrand disease. *Br J Haematol* 2000; 111: 1236-1239.

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 41% of the women with type 3 VWD did not report menorrhagia; one wonders whether some women had mutations resulting in a lesser bleeding tendency than is usually associated with type 3 VWD, or, was this group loathe to claim to have medical problems.*)

Kujovich, JL. Von Willebrand disease and pregnancy. *J Thromb Haemost* 2005; 3:246-253. Review, 80 references.

### Epidural/spinal anesthesia for delivery

*I found no series, so listed some case reports.*

Cohen S, Daitch JS, Amar D, Goldiner PI. Epidural analgesia for labor and delivery in a patient with von Willebrand's disease. *Reg Anesth* 1989; 14:95-97.

A woman with a diagnosis of VWD, no baseline laboratory tests given, had epidural anesthesia for delivery. DDAVP was given 50 minutes before delivery. No excessive bleeding occurred.

Milaskiewicz RM, Holdcroft A, Letsky E. Epidural anaesthesia and von Willebrand's disease. *Anaesthesia* 1990; 45:462-464.

A woman with VWD had, before pregnancy, baseline levels of 42% FVIII, 30% VWF:Ag, and a BT > 15 minutes. At 36 weeks gestation she had 108% FVIII, 10% VWF:RCo and a normal BT. She had epidural anesthesia for a caesarian delivery, performed without blood products or DDAVP. She developed an abdominal wall hematoma but had no problems from the anesthesia.

Jones BP, Bell EA, Maroof M. Epidural labor analgesia in a parturient with von Willebrand's disease type IIA and severe preeclampsia. *Anesthesiology* 1999; 90:1219-1220.

A woman with a history of type 2A VWD had, at 35 weeks gestation, 115% FVIII, 122% VWF:Ag and <25% VWF:RCo. FVIII-VWF concentrate was given before epidural anesthesia and at once to twice daily intervals until the 6<sup>th</sup> post-delivery day, after delivery. She had no bleeding complications.

### Diagnostic tests

#### Reviews on choice of diagnostic tests

Favaloro EJ. Laboratory assessment as a critical component of the appropriate diagnosis and sub-classification of von Willebrand's disease. *Blood Reviews* 1999; 13:185-204. (*Review, 81 refs, useful tables and algorithms reflecting tests used in Australia.*)

Federici AB. Diagnosis of von Willebrand disease. *Haemophilia* 1998; 4:654-60  
*A simpler algorithm than that of Dr. Favaloro is presented, reflecting tests available in Italy and western Europe.*

Ingerslev J, Gursel T. Diagnosis of Von Willebrand disease. *Haemophilia* 1999; 5 (suppl 2): 50-56. *This concise review reflected experience in Scandinavia.*

Favaloro EJ, Nair SC, Forsyth CJ. Collection and transport of samples for laboratory testing in von Willebrand's disease (VWD): Time for a reappraisal? *Thromb Haemost* 2001; 86:1589-1590.

More than 90% of samples at the author's referral center for VWD testing had been 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 abnormality. 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.

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*

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 that multimer analysis then be performed, 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 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.

### 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.

"A small cut is made in the lobe of the ear. At half-minute intervals the blood is blotted up on absorbent paper. This gives a series of blots of gradually decreasing size. Each blot represents one half-minute's outflow of blood. The rate of decrease in the size of the blots shows the rate of decrease of the hemorrhage. The cut should be made of such a size that the first half minute's outflow of blood makes a blot 1 or 2 cm. in diameter. The total duration of such a hemorrhage is called the bleeding time." He made cuts of different sizes and showed that, within reasonable limits, "the duration of a hemorrhage does not depend on the size of the cut." Illustrations show that larger cuts resulted in larger initial blots but duration of bleeding was the same with large and small cuts. Dr. Duke found markedly prolonged bleeding times in patients with severe thrombocytopenia but no prolongation in a patient with hemophilia.

Ivy AC, Shapiro PF, Melnick P. The bleeding tendency in jaundice. *Surg Obstet Gynec* 1935; 60:781-4.

Patients with liver disease who had normal BTs by Duke's method often bled excessively with surgical procedures. To make the BT more sensitive, the authors put a blood pressure cuff around the upper arm, inflated to 40 mm Hg, and punctured the forearm "over the pronator muscles" with a stylet penetrating 2.5 mm. After testing 115 normal persons, they set the upper limit of normal for the modified BT at 4 minutes, not much different from the 3-minute upper limit with the Duke BT. Jaundiced patients tended to have long BTs by the modified method.

Ivy AC, Nelson D, Bucher G. The standardization of certain factors in the cutaneous "venostasis" bleeding time technique. *J Lab Clin Med* 1941; 26:1812-22

The above test was further defined. A well-sharpened thin-bladed scalpel protruding 3 mm from a lancet was used to make the

"punctures" in the supine forearm with a blood-pressure cuff around the upper arm at 40 mm Hg. After the puncture, drops of blood were to be collected gently at 10-second intervals on filter paper, taking care that the paper did not re-open the wound. In tests on 88 subjects, the maximum BT was defined (including 99.5% of results) at 240 seconds (4 minutes). Their major problem with the test was that about 20% of the first and the second punctures on their subjects did not bleed at all. With repeated stabs, they could get some punctures that bled. They advised averaging the results of three punctures that actually bled. (*When I was introduced to the Ivy BT as a Fellow in the early 1960's, we did indeed stab or "puncture" the forearm with a pointed scalpel blade, and not all punctures bled.*)

Quick AJ. Salicylates and bleeding: The aspirin tolerance test. *Am J Med Sci* 1966; 252:265-269.

Two hours after ingestion of 1.3 grams of aspirin, a small but significant increase in the Duke BT was demonstrable in most of ten normal subjects. A woman and her two daughters, suspected of having von Willebrand disease, had normal Duke BTs prior to aspirin ingestion and prolonged times (and more profuse bleeding) afterwards. (*Quick advocated testing the BT after aspirin to increase the sensitivity of the BT in patients suspected of having VWD.*)

Quick AJ. Acetylsalicylic acid as a diagnostic aid in hemostasis. *Amer J Med Sci* 1967; 254:392-397.

Four patients with symptoms suggesting VWD had normal BTs; two hours after ingesting 0.65 grams of aspirin the BTs were mildly increased 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.

Quick AJ. The Minot-von Willebrand syndrome. *Amer J Med Sci* 1967; 253: 520-530.

The aspirin tolerance test was performed on 43 of his 50 most recent VWD patients. All chosen subjects had baseline Duke BTs of no more than five minutes. After aspirin, the BT was 7 minutes or greater in 24 subjects, and was 10 minutes or greater in 10 subjects. *A picture shows the drops of blood individually collected from incisions onto filter paper; seriously-affected patients have larger drops of blood than mildly-affected ones. Copious flow of blood, as well as duration of flow, may be suggestive of VWD.*

Mielke CH Jr, Kaneshiro MM, Maher IA, Weiner JM, Rapaport SI. The standardized normal Ivy bleeding time and its prolongation by aspirin. *Blood* 1967; 34:204-215.

The Ivy BT incision was standardized by using 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 BTs were repeated. The mean baseline BT was 5 minutes, range 2.5 to 10. The mean BT after placebo was 5.5 min and after ASA was 9.5 min (maximum 16 min.) The post-aspirin BT exceeded 10 min 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.

Kaneshiro MM, Mielke CH Jr, Kasper CK, Rapaport SI. Bleeding time after aspirin in disorders of intrinsic clotting. *N Engl J Med* 1969; 281: 1039-42.

The baseline Ivy BT was within normal limits in nine of 11 patients with severe hemophilia A and eight with severe hemophilia B and after aspirin ingestion was prolonged (beyond the normal post-aspirin range established in the above paper) in 12 patients, of whom seven 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 related 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.

Weiss HJ. Relation of von Willebrand factor to bleeding time. *New Engl J Med* 1974; 291:420

In 23 patients with VWD, low levels of plasma VWF:RCo were well-correlated with prolonged BTs. Twelve patients with more than 20% VWF:RCo had normal BTs whereas patients with lower levels of VWF:RCo had moderately to severely prolonged BTs.

### Platelet adhesiveness

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

Salzman EW. Measurement of platelet adhesiveness: A simple in vitro technique demonstrating an abnormality in von Willebrand's disease. *J Lab Clin Med* 1963; 62:724-735.

A platelet count was made on anticoagulated venous blood obtained by phlebotomy. 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 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 range in 45 normal subjects 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*).

Strauss HS, Bloom GE. Von Willebrand's disease: Use of a platelet-adhesiveness test in diagnosis and family investigation. *New Engl J Med* 1965; 273:171-181.

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, and fairly specific.

Meyer D, Larrieu MJ. Von Willebrand's factor and platelet adhesiveness *Clin Pathol* 1970; 23:228-31.

Salzman's method was modified for *in vitro* use by first drawing a fluid (buffer, plasma, cryoprecipitate or FVIII concentrate) through the tubing to coat the glass bead. Blood from patients with VWD then was allowed to flow through the apparatus. VWD patients all had baseline platelet adhesiveness levels below 5%. A definite increase in platelet adhesiveness was seen if the beads were pre-coated with normal plasma (mean 18%) or hemophilic plasma (15%) but not if they were pre-coated with VWD plasma. A greater increase was seen if the beads were pre-coated with cryoprecipitate (mean 25%) or intermediate-purity factor VIII concentrate (35.8%). Prior *in vivo* infusion of intermediate-purity factor VIII concentrate also corrected the platelet adhesiveness in five patients with VWD. These results suggested the existence in normal and hemophilia plasma of a factor (*VWF, not yet clearly recognized*) transferable to glass beads and capable of inducing retention of Willebrand platelets.

Bouma BN, Sixma JJ, De Graaf S, Wiegerinck Y, van Mourik JA, Mochtar UA. Factor-VIII antigen and platelet retention in a glass bead column. *Br J Haematol* 1973; 25: 645-656.

Cryoprecipitate made from normal and hemophilia A plasma corrected the abnormal platelet adhesiveness found in VWD. "Factor-VIII-related-antigen" (VWF:Ag) was presumed to be responsible for the correction (*that is, rather than any platelet characteristic*).

## Platelet function analyzers

Kundu SK, Heilman EJ, Sio R, Garcia C, Davidson RM, Ostgaard RA. Description of an in-vitro platelet function analyzer: PFA-100®. *Semin Thromb Hemost* 1995; 21 (suppl 2):106-112.

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<sup>-1</sup>. When a stable platelet plug forms, it closes the aperture. The time required for aperture closure is called the "closure time", CT. Each test uses a disposable "test cartridge" containing the reservoir for the blood sample, the capillary tube, membrane, aperture and cup for blood that has passed through the assembly. Different cartridges are used to test the CT with epinephrine versus the CT with ADP. The instrument was developed to mimic the BT as a more-standardized test of platelet-plug formation.

Mammen EF, Alshameeri RS, Comp PC. Preliminary data from a field trial of the PFA-100® system. *Semin Thromb Hemost* 1995; 21 (suppl 2): 113-121.

Testing of citrated whole blood must be performed within five hours after drawing the sample, and, in the interval, the sample must be kept at room temperature. In normal subjects, CTs ranged from 77-133 seconds for collagen/ADP cartridges and 98- 185 seconds for collagen/epinephrine cartridges.

Fressinaud E, Veyradier A, Sigaud M, Boyer-Neumann C, Le Boterff C, Meyer D. Therapeutic monitoring of von Willebrand disease: interest and limits of a platelet function analyser at high shear rates. *Br J Haematol* 1999; 106:277-81.

After infusion of DDAVP, the CT became normal in all patients with type 1 VWD (n=23) and in most but not all of those with type 2 (n=11). The CT in type 3 (n=4) remained prolonged after infusion of FVIII-VWF concentrate. The very high shear conditions in the PFA-100® make it sensitive to the contribution of platelet VWF and to ultralarge VWF multimers. The CT can be used to follow the response of therapy in patients with normal platelet VWF.

Fressinaud E, Veyradier A, Truchaud F, Martin I, Boyer-Neumann C, Trossaert M, Meyer D. Screening for von Willebrand Disease with a new analyzer using high shear stress: A study of 60 cases. *Blood* 1998; 91:1325-1331.

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. 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. Results were as follows:

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

Cattaneo M, Federici AB, Lecchi A, Agati B, Lombardi R, Stabile F, Bucciarelli P. Evaluation of the PFA-100® system in the diagnosis and therapeutic monitoring of patients with von Willebrand disease. *Thromb Haemost* 1999; 82:35-9

In Italy, CTs were measured in 52 patients with VWD and in 40 normal persons. The sensitivity of the BT in VWD was only 65% whereas the sensitivity of the PFA-100® was 88% with the ADP cartridge and 87% with the epinephrine cartridge. DDAVP did not shorten the CT in type 2A VWD or in type 1 VWD with low platelet VWF. Use of FVIII-VWF concentrate in type 3 VWD slightly shortened the CT.

Favaloro EJ, Facey D, Henniker A. Screening for von Willebrand's disease: Use of a novel Platelet Function Analyser (PFA-100®) with high sensitivity to disturbances in von Willebrand disease. *Am J Hematology* 1999; 62:165-174

In Australia, nine patients with VWD (three type 1, one 2A, four 2B, one type 3) had prolonged CTs with both cartridges. The epinephrine cartridge was more sensitive to VWD, but less specific for VWD, than the ADP cartridge. The ADP cartridges were recommended for screening purposes, if the cost of using two cartridges per patient must be avoided.

Favaloro EJ. Utility of the PFA-100® for assessing bleeding disorders and monitoring therapy: a review of analytical variables, benefits and limitations. *Haemophilia* 2001; 7:170-179. (Review, 43 refs)

No great difference in CTs were found between genders and among age groups in normal persons. A slightly elevated CT was found in smokers compared to non-smokers. The CT was not influenced by the presence of moderate amounts of heparin in the sample. Any drug that depressed platelet function, such as aspirin, could prolong the CT. Low hematocrits, low white blood cell counts and low platelet counts were associated with prolonged CTs. The CT was not sensitive to fibrinogen deficiencies or defects, nor to low FVIII. The test had varying levels of sensitivity to disturbances in platelet function but was highly sensitive to disturbances in VWF function. The CT was not universally abnormal in type 1 VWD. When used to monitor therapy, the CT was not always corrected. The PFA-100® was used as a screening test for VWF and for inherent or drug-induced platelet function abnormalities.

Cariappa R, Wilhite TR, Parvin CA, Luchtman-Jones L. Comparison of the PFA-100® and bleeding time testing in pediatric patients with suspected hemorrhagic problems. *J Pediatr Hematol Oncol* 2003; 25: 474-479.

In the detection of qualitative platelet abnormalities, the epinephrine cartridge CT offered 100% sensitivity and 97% specificity, compared with 37% and 88% respectively for the BT. For children with VWD, the sensitivity of the epinephrine cartridge CT was 100% and that of the bleeding time was only 17%.

Posan E, McBane RD, Grill DE, Motsko CL, Nichols WL. Comparison of PFA-100® testing and bleeding time for detecting platelet hypofunction and von Willebrand disease in clinical practice. *Thromb Haemost* 2003; 90:483-490.

CTs and Ivy BTs were used to evaluate 346 outpatients referred for testing for a bleeding disorder. The sensitivity of the CT in 34 patients with VWD was significantly better than the BT. The sensitivity of the CT was comparable but not superior to the BT in 31 patients with platelet hypofunction.

Nitu-Whalley IC, Lee CA, Brown Sa, Riddell A, Hermans C. The role of the platelet function analyser (PFA-100®) in the characterization of patients with von Willebrand's disease and its relationships with von Willebrand factor and the ABO blood group. *Haemophilia* 2003; 9:298-302.

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. The CT did not vary according to ABO blood groups.

## Ristocetin induced platelet aggregation (RIPA)

Howard MA, Firkin BG. Ristocetin - A new tool in the investigation of platelet aggregation. *Thromb Diath Haemorrh* 1971; 26:362-369.

The ability of ristocetin to aggregate platelets in platelet-rich plasma in vitro was described. (The test described, the first used with

*ristocetin*, is *ristocetin-induced platelet aggregation*, 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.

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.

RIPA was quantitated by measuring the rate of aggregation, that is, the slope of the line of aggregation. (Aggregation was measured by following the change in optical density of the platelet suspension in a cuvette. The optical density was recorded as a line drawn on moving paper as is still done today.) The maximum rate of aggregation was more useful than the maximum extent of aggregation in diagnosing VWD.

### Ristocetin cofactor (VWF:RCo)

Weiss HJ, Hoyer LW, Rickles FR, Varma A, Rogers J. Quantitative assay of a plasma factor deficient in von Willebrand's disease that is necessary for platelet aggregation: Relationship to factor VIII procoagulant activity and antigen content. *J Clin Invest* 1973; 52:2708-2716

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.

Allain JP, Cooper HA, Wagner RH, Brinkhous KM. Platelets fixed with paraformaldehyde: a new reagent for assay of von Willebrand factor and platelet aggregating factor. *J Lab Clin Med* 1975; 85: 318-328.

Normal platelets fixed in formalin proved to be a stable reagent for the ristocetin cofactor test.

Brinkhous KM, Graham JE, Cooper HA, Allain JP, Wagner RH. Assay of von Willebrand factor in von Willebrand's disease and hemophilia: Use of macroscopic platelet aggregation test. *Thromb Res* 1975; 6:267-272.

Using formalin-fixed platelets, the authors measured ristocetin cofactor (VWF:RCo) 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 was, and is, suitable for minimally-equipped laboratories around the world.)

Brinkhous KM, Read MS. Preservation of platelet receptors for platelet aggregating factor/von Willebrand factor by air drying, freezing or lyophilization: New stable platelet preparations for von Willebrand factor assays. *Thromb Res* 1978; 13:591-597.

Platelets fixed in formalin for use in the ristocetin cofactor assay were stable if frozen or lyophilized (making the assay for VWF:RCo more convenient, and making commercial kits possible.)

Ramsey R, Evatt BE. Rapid assay for von Willebrand factor activity using formalin-fixed platelets and microtitration technic. *Am J Clin Path* 1979; 72:996-9.

Platelet aggregation was easier to observe in a microtiter plate than in test-tubes, (a suggestion suitable for testing in simple laboratories.)

Miller CH, Platt SJ, Daniele C, Kaczor D. Evaluation of two automated methods for measurement of the ristocetin cofactor activity of von Willebrand factor. *Thromb Haemost* 2002; 88: 56-59.

An automated coagulation analyzer which stirs the contents of the cuvette and reads optical density was used to assay VWF:RCo with good reproducibility. (This analyzer sounds practical for a clinical laboratory.)

### Use of Botrocetin

Read MS, Shermer RW, Brinkhous KM. Venom coagglutinin: An activator of platelet aggregation dependent on von Willebrand factor. *Proc Natl Acad Sci* 1978; 75:4514-4518.

An array of snake venoms were tested for platelet-aggregating ability dependent on Von Willebrand factor (at a time when ristocetin was banned from the US market, even as a reagent.) Such ability was found predominantly in the venom of the genus *Bothrops*. (The new reagent was called *Botrocetin*.)

Brinkhous KM, Read MS, Fricke WA, Wagner RH. Botrocetin (venom coagglutinin): Reaction with a broad spectrum of multimeric forms of factor VIII macromolecular complex. *Proc Natl Acad Sci* 1983; 80:1463-1466.

Botrocetin was able to induce platelet aggregation in platelet-rich plasma lacking HMW multimers, whereas ristocetin requires HMW multimers. Botrocetin-induced aggregation correlates with the level of VWF:Ag. (Botrocetin did not substitute for ristocetin because it was not equally sensitive in type 2 VWD patients. It is now primarily a research tool.)

### Monoclonal antibody assays of VWF functional sites

Goodall AH, Jarvis J, Chand S, Rawling E, O'Brian DP, McGraw A, Hutton R, Tuddenham EGD. An immunoradiometric assay for human factor VIII/von Willebrand factor (VIII:vWF) using a monoclonal antibody that defines a functional epitope. *Br J Haematol* 1985; 59:565-577.

A mouse monoclonal antibody that bound to a VWF epitope so as to totally neutralize ristocetin-induced aggregation of platelet-rich plasma and to inhibit platelet adhesion at high flow rates. It could be used in one-stage, fluid-phase immunoradiometric assays. In VWD the results correlate with VWF:RCo rather than with VWF:Ag in those types of VWD with discrepant levels of VWF:Ag and VWF:RCo. The test was advocated for detection of functional deficits of VWF.

Murdock PJ, Woodhams BJ, Matthews KB, Pasi KJ, Goodall AH. Von Willebrand factor activity detected in a monoclonal antibody-based ELISA: an alternative to the ristocetin cofactor platelet agglutination assay for diagnostic use. *Thromb Haemost* 1997; 78:1272-1277.

Another monoclonal antibody was produced that bound to a VWF epitope involved in its interaction with GPIIb/IIIa. A solid phase ELISA test was developed using that antibody as capture antibody. The monoclonal-antibody-based ELISA had advantages of sensitivity and reproducibility over the ristocetin cofactor test. (This test is used at the Royal Free Hospital in London. Some other laboratories had difficulty with the assay.)

### VWF binding to collagen (VWF:CB)

Brown JE, Bosak JO. An ELISA test for the binding of von Willebrand antigen to collagen. *Thromb Res* 1986; 43:303-311.

Microtiter plates were coated with type 1 bovine tendon collagen. Plasma in various dilutions was incubated in wells in the plates. The amount of VWF bound to the collagen was detected with antibodies to VWF. With normal plasma, and with plasma from patients with type 1 VWD, VWF:Ag levels correlated with VWF:CB. In patients with type 2A VWD, plasma levels of VWF:Ag were much higher than VWF:CB. VWF:CB was suggested as a functional test instead of VWF:RCo.

Duggan MG, DiMichele DM, Christian MJ, Fink LM, Hathaway WE. Collagen-binding of von Willebrand's factor antigen in the classification of von Willebrand's disease. *Am J Clin Pathol* 1987; 88:97-102.

An assay for VWF:CB used commercially-available bovine dermal collagen, 95% type I and 5% type III. Patient plasma was incubated with a solution of collagen in a test-tube. The supernatant was assayed for VWF:Ag. In 48 normal persons, a mean of 85.4% of the VWF bound to collagen. In four persons with type 1 VWD, 80.8% of the VWF bound to collagen but in eight persons with type 2 VWD only 32.3% of the VWF bound to collagen. This simple assay was reproducible in their hands.

Favaloro EJ, Grispo L, Exner T, Koultis J. Development of a simple collagen based ELISA assay aids in the diagnosis of, and permits sensitive discrimination between type I and type II von Willebrand's disease. *Blood Coagul Fibrinolysis* 1991; 2:285-291.

In Australia, the test of Brown and Bosak was modified, using an ELISA method. Dilutions of normal or patient plasma were put in wells in glass plates coated with type 1 equine collagen. The plates were

incubated and washed, then further incubated with a horseradish peroxidase labelled rabbit antiserum to human VWF and then further incubated with peroxidase substrate to allow color development. The VWF:CB assay appeared to be more sensitive and more reproducible than the VWF:RCo for diagnosing type 2 VWD and became the preferred functional test in Australia. Laboratory values according to VWD type (simplified from their table 1) were as follows:

Subjects	N	VWF:Ag, %, mean	VWF:RCo, %, mean	VWF:CB, %, Mean
Normal	42	97.9	102.5	106.9
VWD Type 1	37	47.3	31.1	60.7
VWD Type 2	16	34.9	11.9	1.6

Favaloro EJ, Henniker A, Facey D, Hertzberg M. Discrimination of von Willebrand's disease (VWD) subtypes: Direct comparison of von Willebrand factor:collagen binding assay (VWF:CBA) with monoclonal antibody (MAB) based VWF-capture systems. *Thromb Haemost* 2000; 84:541-547.

A commercial test kit for VWF activity, utilizing a monoclonal antibody against the VWF A1 domain, was compared to the efficacy of locally-produced similar monoclonal antibodies and to the collagen-binding assay. Test results with the monoclonal-antibody kit tended to be higher and similar to levels of VWF:Ag rather than to the lower levels of VWF:RCo in type 2 VWD patients. In the authors' lab, the VWF:CB test was more useful in discriminating type 2 from type 1 patients.

Casonato A, Pontara E, Bertomoro A, Sartorello F, Cattini MG, Girolami A. Von Willebrand factor collagen binding activity in the diagnosis of von Willebrand disease: an alternative to ristocetin cofactor activity? *Br J Haematol* 2001; 112:578-583.

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

Subjects	N	VWF:RCo, %, mean	VWF:CB, %, mean
Normal	50	102.1	99.7
VWD type 1	30	32.8	35.2
VWD type 2A	10	33.0	7.4
VWD type 2B	12	18.9	8.1

Favaloro EJ. Von Willebrand factor collagen-binding (activity) assay in the diagnosis of von Willebrand disease: A 15-year journey. *Sem Thromb Haemost* 2002; 28:191-202. Review, 94 references.

### VWF binding to factor VIII (VWF:FVIII)

Nishino M, Girma JP, Rothschild C, Fressinaud E, Meyer D. New variant of von Willebrand disease with defective binding to factor VIII. *Blood* 1989; 74: 1591-9.

A monoclonal antibody to VWF was coated onto wells of microtitration 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% respectively, VWF:Ag of 39 and 53%, VWF:RCo 39 and 54%. Binding of purified FVIII to their VWF was markedly decreased.

Casonato A, Pontara E, Zerbini P, Zucchetto A, Girolami A. The evaluation of factor VIII binding activity of von Willebrand factor by means of an ELISA method: significance and practical implications. *Am J Clin Pathol* 1998; 109:347-352.

A new 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:FVIII 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:

Subjects	FVIII	VWF:Ag	VWF:RCo	VWF:FVIII
Normal	60-160	60-160	60-130	0.6-1.3
Homozygotes	8.3-8.7	40-48.5	48.7-49.2	0.17-0.21
Heterozygotes	55-76	29.3-33.1	21.9-38	1.2-1.3

Caron C, Mazurier C, Goudemand J. Large experience with a factor VIII binding assay of plasma von Willebrand factor using commercial reagents. *Br J Haematol* 2002; 117:716-718.

An ELISA was described, to be performed entirely with inexpensive reagents available commercially (rather than the home-made reagents used by Nishino et alia and others) to test VWF binding to FVIII.

Taylor SL, Bromidge E, Savidge GF, Alhaq A. Evaluation of an automated screening assay for von Willebrand disease type 2N. *Clin Lab Haematol* 2002; 24:369-375.

An ELISA assay measuring VWF:FVIII binding in parallel with VWF:Ag uses monoclonal capture and detection antibodies and is performed in a robotic microtiter plate processor with the capacity to screen large numbers of samples.

### Von Willebrand factor antigen (VWF:Ag)

Zimmerman TS, Hoyer LW, Dickson L, Edgington TS. Determination of the von Willebrand's disease antigen (factor VIII-related antigen) in plasma by quantitative immunoelectrophoresis. *J Lab Clin Med* 1975; 86:152-159.

VWF:Ag was quantified by electrophoresis of 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. (This method was used before ELISA methods were developed.)

Over J, Vlooswijk HAA, Sixma JJ. Assay of F. VIII-related antigen in a variant of von Willebrand's disease. *Thromb Haemost* 1977; 37:367-370.

With the Laurell method, measuring the height of precipitin rockets, one may over-estimate the amount of VWF:Ag in patients who lack large multimers. Fast-traveling low-molecular-weight VWF:Ag may mark a rocket the same height as normal, albeit fainter.

Ruggeri ZM, Mannucci PM, Jeffcoate SL, Ingram GL. Immunoradiometric assay of factor VIII related antigen, with observations in 32 patients with von Willebrand's disease. *Br J Haematol* 1976; 33:221-232.

A solid-phase immunoradiometric assay allowed measurement of low levels of VWF:Ag. In 17 patients with severe symptoms and unmeasurable levels of VWF:RCo, VWF:Ag ranged from 1.3% to < 0.25 %

Silveira AMV, Yamamoto T, Adamson L, Hessel B, Blomback B. Application of an enzyme-linked immunosorbent assay (ELISA) to von Willebrand factor (vWF) and its derivatives. *Thromb Res* 1986; 43:91-102.

In an ELISA, microtiter plates were coated with a goat F(ab')<sub>2</sub> antibody to VWF. After the VWF from a plasma sample was bound to the plate, the amount bound was quantified using a horse-radish peroxidase-labelled goat Fab' antibody to VWF. The test was more sensitive than previous ELISA methods to traces of VWF. Another method of quantification, with monoclonal antibodies to multimeric and to reduced forms of VWF, allowed estimation of the proportion of multimers of various sizes.

## Multimers

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 platelets. *J Clin Invest* 1980; 65:1318-1325. (see historical section)

Miller MA, Palascak JE, Thompson MR, Martelo OJ. A modified SDS agarose gel method for determining von Willebrand factor multimers using commercially available reagents. *Thromb Res* 1985; 39: 777-780.

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

Mannucci PM, Abildgaard CF, Gralnick HR, Hill FGH, Hoyer LW, Lombardi R, Nilsson IM, Tuddenham E, Meyer D. Multicenter comparison of von Willebrand factor multimer sizing techniques. *Thromb Haemost* 1985; 54:873-877

Plasma from six patients with various multimer abnormalities were studied in seven laboratories in low-resolution and high-resolution gels. Low-resolution gels could distinguish a normal set of multimers, as in type 1 VWD, from a loss of high-molecular weight multimers (type 2), and were used as a screening test. High-resolution gels are needed to identify alterations of the internal structure of multimers.

Aihara M, Sawada Y, Ueno K, Morimoto S, Yoshida Y, de Serres M, Cooper HA, Wagner RH. Visualization of von Willebrand factor multimers by immunoenzymatic stain using avidin-peroxidase complex. *Thromb Haemost* 1986; 56: 263-267.

To avoid using radio-isotopes for labeling multimers, an avidin-biotin-peroxidase complex was added to the multimer-bearing gel. Peroxidase activity was detected by chloro-naphthol, marking bands sharply and displaying triplet structure.

Tomita Y, Harrison J, Abildgaard CF. Von Willebrand factor multimer analysis using a sensitive peroxidase staining method. *Thromb Haemost* 1989; 62:781-3

SDS-agarose electrophoresis followed by blotting to a nitrocellulose membrane and a sensitive peroxidase staining method gave resolution comparable to techniques using radio-isotopes.

Raines G, Aumann H, Sykes S, Street A. Multimeric analysis of von Willebrand factor by molecular sieving electrophoresis in sodium dodecyl sulphate agarose gel. *Thromb Res* 1990; 60: 201-212. *The excellent discussion section in this article is reasonably accessible to the general reader.*

"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. 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."

Smejkal GB, Shainoff JR, Kottke-Marchat KM. Rapid high-resolution electrophoresis of multimeric von Willebrand Factor using a thermopiled gel apparatus. *Electrophoresis* 2003; 24: 1482.

Agarose gel electrophoresis of VWF was performed with an apparatus that monitors and precisely controls gel temperature. With maintenance of the temperature at 10° C, the electrophoresis was completed in 20 minutes with 250 V. (This method is much faster than those previously described, which required many hours for the electrophoresis phase, in addition to the time required for washing and staining.)

## TREATMENT

### Reviews

Federici AB, Sacco R, Stabile F, Carpenedo M, Zingaro E, Mannucci PM. Optimizing local therapy during oral surgery in patients with von Willebrand disease: effective results from a retrospective analysis of 63 cases. *Haemophilia* 2000; 6:71-77.

For oral surgery, all patients received tranexamic acid on the day before and for seven days afterwards. If three or more teeth were extracted, fibrin glue was applied locally. DDAVP was given (0.3 ug/kg subcutaneously, 60 min 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.

Local therapy only (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 local 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 local therapy were given on 21 occasions to patients with type 2 or 3 VWD; only one had post-extraction bleeding.

Nitu-Whalley IC, Griffioen A, Harrington C, Lee CA. Retrospective review of the management of elective surgery with desmopressin and clotting factor concentrates in patients with von Willebrand disease. *Am J Hematol* 2001;66:280-4

In a ten-year period, 27 patients 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 usually was also given for mucosal surgery. Hemostasis was excellent in 91% of surgical procedures managed with DDAVP. 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. Duration of treatment averaged 10 days for major operations, four for minor operations, six for ENT procedures, 7 for deliveries and one for dental procedures.

Siegel JE, Kouides PA. Menorrhagia from a haematologist's point of view. Part II: management. *Haemophilia* 2002; 8:339-347. *An excellent current 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 these agents 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.

Pasi KJ, Collin PW, Keeling M, Brown SA, Cumming AM, Dolan GC, Hay CRM, Hill FGH, Laffan M, Peaker IR. Management of von Willebrand disease: a guideline from the UK Haemophilia Centre Doctors' Organization. *Haemophilia* 2004; 10:218-231. *An authoritative review with dosage suggestions.*

Rodeghiero F, Castaman C. Treatment of von Willebrand disease. *Semin Hematol* 2005; 42:29-35. *A review*

Federici AB. Management of inherited von Willebrand disease in 2006. *Semin Thromb Haemost* 2006; 32:616-620. *A current review.*

Federici AM, Mannucci PM. Management of inherited von Willebrand disease in 2007. *Ann Med* 2007; 39:34-358. *A review, also addressing prophylaxis.*

### Anti-fibrinolytic agents

Reid WO, Lucas ON, Francisco J, Geisler PH, Easley AJ. The use of epsilon-aminocaproic acid in the management of dental extractions in the hemophiliac. *Amer J Med Sci* 1964, 248:184-188.



Zealand Med J 1980; 92: 375-376.

DDAVP was given intravenously at 12-hour intervals, with EACA, to five patients with VWD for the following procedures: a tonsillectomy and adenoidectomy, a cyst excision, an extraction of a single tooth and two extractions of four wisdom teeth. BTs were corrected; levels of FVIII and VWF:Ag rose markedly. No patient needed blood products.

Mannucci PM, Canciani MT, Rota L, Donovan BS. Response of factor VIII/von Willebrand factor to DDAVP in healthy subjects and patients with haemophilia A and von Willebrand's disease. *Br J Haematol* 1981; 47:283-293.

At a dose of 0.3 ug/kg intravenously, DDAVP produces 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. In some normal subjects and 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 ("tachyphylaxis").

Warrier AI, Lusher JM. DDAVP: A useful alternative to blood components in moderate hemophilia A and von Willebrand disease. *J Ped* 1983; 102:228-232.

The effect of intravenous DDAVP (0.2 ug/kg, less than used in later years) was studied in three normal persons, 18 persons with VWD and 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. 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.

Barnhart MI, Chen S, Lusher JM. DDAVP: Does the drug have a direct effect on the vessel wall? *Thromb Res* 1983; 21:239-253.

A normal human umbilical vein perfusion model was used to study DDAVP effects. 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 as a 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 sub-endothelium, encouraging platelet binding.

Sakariassen KS, Cattaneo M, vd Berg A, Ruggeri ZM, Mannucci PM, Sixma JJ. DDAVP enhances platelet adherence and platelet aggregate growth on human artery subendothelium. *Blood* 1984; 64:229-236.

Human umbilical artery, denuded of the endothelial layer, was perfused with whole blood or its 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, infused with DDAVP, was much enhanced over baseline. Platelet adherence was unaffected or only slightly improved after infusion of DDAVP into persons with type 2A VWD. Platelet adherence decreased after infusion of DDAVP into persons with type 2B VWD.

De la Fuente B, Kasper CK, Rickles FR, Hoyer LW. Response of patients with mild and moderate hemophilia A and von Willebrand disease to treatment with desmopressin. *Ann Intern Med* 1985; 103:6-14

Intravenous DDAVP, 0.3 ug/kg, was given to 21 VWD patients. Levels of FVIII increased more than levels of VWF:Ag or VWF:RCo. A prolonged BT was fully corrected in eight patients and partially corrected in two. Individuals tended to have similar responses to DDAVP on each occasion of its use (if separated by a few days) and members of the same family tended to have similar levels of response to DDAVP.

Gralnick HR, Williams SB, McKeown L, Rick ME, Maisonneuve P, Jenneau C, Sultan Y. DDAVP in type IIa von Willebrand disease. *Blood* 1986; 67: 465-468

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. Although these 2A patients respond well to DDAVP, the VWF is easily lysed.

Rodeghiero F, Castaman G, Di Bona E, Ruggeri M. Consistency of responses to repeated DDAVP infusions in patients with von Willebrand's disease and hemophilia A. *Blood* 1989; 74:1997-2000.

DDAVP infusions were given to 22 patients with VWD and 10 with mild to moderate hemophilia A on two occasions, at least a month apart. There were 14 patients with type 1 platelet-normal, two with type 1 platelet-low, five with type 2M, and one with un-characterized type 2 VWD. Very similar responses of FVIII and BT were seen from one occasion to the next. More than one person with VWD from five families were tested and within-family consistency also was seen. The authors concluded that one test dose of DDAVP sufficed to predict a given patient's response to the drug. (Only one patient, with VWD type 1 platelet-low, might be considered to have had an inadequate, minimal response.)

Mannucci PM, Bettega D, Cattaneo M: Patterns of development of tachyphylaxis in patients with hemophilia and von Willebrand disease after repeated doses of desmopressin (DDAVP). *Br J Haematol* 1992; 82:87-93

Intravenous DDAVP, at 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. On average, 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. In contrast to the plasma factor response, prolonged BTs 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 in a satisfactory range.)

Sultan Y, Loyer F, Venot A. Impaired fibrinolytic response to DDAVP in patients with von Willebrand's disease. *Nouv Rev Fr Hematol* 1992; 34:55-60.

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."

Wun T, Paglieroni TG, Lachant JA. Desmopressin stimulates the expression of P-selectin on human platelets in vitro. *J Lab Clin Med* 1995; 126:401-409.

Platelet-rich plasma was incubated with dilutions of DDAVP. Expression of the platelet activation-dependent antigen known as CD 62 or P-selectin was stimulated at pharmacologic levels of DDAVP. This may partly explain the nonspecific clot-promoting action of DDAVP.

Nolan B, White B, Smith J, O'Reilly C, McMahon C, Fitzpatrick B, Smith OP. Desmopressin: therapeutic limitations in children and adults with inherited coagulation disorders. *Br J Haematol* 2000; 109:865-869.

Over a three-year period, all patients presenting with mild hemophilia A, type 1 VWD, or platelet function disorders had a test dose of DDAVP. Plasma levels of FVIII, VWF:Ag and VWF:RCo reached normal levels (good response) in 82 of 91 VWD patients tested. Baseline levels of these factors were higher in those 82 patients who responded well to DDAVP (mean 69% FVIII, 47% VWF:Ag and 45% VWF activity measured with an ELISA test) versus those nine who did not respond well (mean 30% FVIII, 12% VWF:Ag and 15% VWF:RCo.)

Federici AB, Mazuric C, Berntorp E, Lee CA, Scharrer I, Goudemand J, Lethagen S, Nitu I, Ludwig G, Hilbert L, Mannucci PM. Biologic response to desmopressin in patients with severe type 1 and type 2 von Willebrand disease:

results of a multicenter European study. *Blood* 2004; 103: 2032-2038.

Patients with more severe forms of type 1 and 2 VWD, e.g. with VWF:RCo of less than 10%, received 0.3 ug DDAVP intravenously. Only one of eight type 1 patients with baseline levels of VWF:RCo under 6% had satisfactory responses to DDAVP (defined as increases in FVIII and VWF:RCo levels of at least three-fold, reaching levels over 30%, plus decrease in prolonged BTs to no more than 12 minutes) whereas 6 of 18 with baseline levels of vWF:RCo of more than 6% had satisfactory responses. Only one of 15 patients with type 2A and 3 of 21 with type 2M had satisfactory responses.

### DDAVP Nasal Spray

Lethagen S, Harris As, Nilsson IM. Intranasal desmopressin (DDAVP) by spray in mild hemophilia A and von Willebrand's disease type I. *Blut* 1990; 60:187-91

The hemostatic effect of DDAVP given intravenously in a dose of 0.3 ug/kg versus that given by nasal spray in a standard dose of 300 ug (150 ug into each nostril) was compared in eight patients with mild hemophilia A and 22 with VWD. A higher rise in FVIII and VWF:Ag was seen with the intravenous dose but shortening of the BT was equivalent with the two routes of administration. The nasal spray was used at home by 23 patients with VWD, most often for nosebleeds and for menorrhagia. Concomitant use of tranexamic acid had been advised for home use, but the frequency of compliance was not stated. (*Patients with nosebleeds use the spray in the non-bleeding side of the nose.*)

Rose EH, Aledort LM. Nasal spray desmopressin (DDAVP) for mild hemophilia A and von Willebrand disease. *Ann Intern Med* 1991; 114:563-568. (n=11)

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.

Lethagen S, Egervall K, Berntorp E, Bengtsson B. The administration of desmopressin by nasal spray: a dose-determination study in patients with mild haemophilia A or von Willebrand's disease. *Haemophilia* 1995; 1:97-102.

The effects of three intranasal doses (300, 450 and 500 ug) of DDAVP were compared in five patients with hemophilia A and 11 with VWD. There were no significant differences in responses 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 and in the others levels of these factors had returned to baseline by 8 hours.

Seremetis SV, Aledort LM. Desmopressin nasal spray for hemophilia A and type 1 von Willebrand disease. (Letter). *Ann Intern Med* 1997; 126:755-745.

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.

Leisinger C, Becton D, Cornell Jr, Cox Gill J. High-dose DDAVP intranasal spray (Stimate) for the prevention and treatment of bleeding in patients with mild haemophilia A, mild or moderate type 1 von Willebrand disease and symptomatic carriers of hemophilia A. *Haemophilia* 2001; 7:258-266.

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, in 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 oc-

curred in a woman with a prior history of hyponatremia.

Kadir RA, Lee CA, Sabin CA, Pollard D, Economides DL. DDAVP nasal spray for treatment of menorrhagia in women with inherited bleeding disorders: a randomized placebo-controlled crossover study. *Haemophilia* 2002; 8:787-793.

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

Holmberg L, Nilsson IM, Borge L, Gunnarsson M, Sjorin E. Platelet aggregation induced by 1-desamino-8-D-arginine vasopressin (DDAVP) in type IIB von Willebrand's disease. *New Engl J Med* 1983; 309:816-821.

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 platelet was demonstrated. The authors recommended that DDAVP not be given to type 2B patients.

Fowler WE, Berkowitz LR, Roberts HR. DDAVP for type IIB von Willebrand disease. *Blood* 1989; 74:1859-1860.

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.

Casonato A, Pontara E, Dannhaeuser D, Bertomoro A, Sartori MT, Zerbinati P, Girolami A. *Blood Coagul Fibrinolysis* 1994; 5:959-964.

Seven patients with type 2B VWD, from four kindreds, were treated successfully for minor surgical procedures with DDAVP. The BT was corrected and levels of FVIII, VWF:Ag and, to a lesser extent, VWF:RCo were adequately raised. Platelet counts had fallen at 30 minutes post-DDAVP but returned to normal by two hours. Use of DDAVP in type 2B VWD may be appropriate.

Castaman G, Rodeghiero F. Desmopressin and type IIB von Willebrand disease. *Haemophilia* 1996; 2:75-77.

The authors review published reports of use of DDAVP in type 2 VWD in which bleeding times and/or platelet counts before and after DDAVP are quoted. In 14 of 20 instances, a marked thrombocytopenia was documented. In only three instances was a prolongation of the BT observed, indeed, the BT was more likely to improve. The authors used DDAVP for hemostasis in three surgical operations and three tooth extractions in patients with type 2B without difficulty. Use of DDAVP in type 2B may be appropriate.

Casonato A, Steffan A, Pontara E, Zucchetto A, Rossi C, De Marco L, Girolami A. Post-DDAVP thrombocytopenia in type 2B von Willebrand disease is not associated with platelet consumption: Failure to demonstrate glycoalbumin increase or platelet activation. *Thromb Haemost* 1999; 81:234-238.

Two markers were studied to elucidate mechanisms of thrombocytopenia after DDAVP in four patients with type 2B VWD. One was plasma levels of platelet glycoalbumin (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, glycoalbumin 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 glycoalbumin levels or platelet expression of P-selectin. The acute thrombocytopenia after DDAVP infusion is not related to platelet activation or consumption.

## DDAVP and hyponatremia

Lowe F, Pettigrew A, Middleton S, Forbes CD, Prentice CRM. DDAVP in haemophilia (letter). *Lancet* 1977; 2:614-615

A man with moderate hemophilia A was treated with DDAVP in a dose of 0.5 ug/kg every 12 hours for five infusions. He developed hyponatremia with a plasma sodium of 124 nmol/l. His only symptom was headache. The authors advised fluid restriction with DDAVP.

Smith TJ, Gill JC, Ambrosio DR, Hathaway WE. Hyponatremia and seizures in young children given DDAVP. *Am J Hematol* 1989;31:199-202.

Four children, under age two years, developed hyponatremia less than 24 hrs after receiving DDAVP in a 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 of the children had grand mal seizures; all recovered. The authors advise fluid restriction and avoidance of hyponatremic solutions after DDAVP in babies.

Shepherd LL, Hutchinson RJ, Worden, EK, Koopmann CF, Coran, A. Hyponatremia and seizures after intravenous administration of desmopressin acetate for surgical hemostasis. *J Ped* 1989; 114:476-472.

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. Fluid restriction was recommended.

Weinstein RE, Bona RD, Altman AJ, Quinn JJ, Weisman SJ, Bartolomeo A, Rickles FR. Severe hyponatremia after repeated intravenous administration of desmopressin. *Am J Hematol* 1989; 32:258-261.

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

Age, years	Number of doses	Serum sodium (mEq/L)	Clinical result
3	3	120	Seizure
4	5	121	Seizure
15	22	118	Obtundation
28	4	118	Agitation, confusion

Humphries JE, Siragy H. Significant hyponatremia following DDAVP administration in a healthy adult. *Am J Hematol* 1993; 44:12-15. *Case report and also review of literature.*

Hyponatremia (121 mEq/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. She received DDAVP for hemostasis during sinus surgery. Neither her oral nor her intravenous fluid intake was excessive. She recovered with restriction of fluids to less than 500 ml/day.

Garcia EBG, Ruitenber A, Madretsma GS, Hintzen RQ. Hyponatraemic coma induced by desmopressin and ibuprofen in a woman with von Willebrand's disease. *Haemophilia* 2003; 9:232-234.

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 at home, with hyponatremia of 121 mmol/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

Bond L, Bevin D. Myocardial infarct in a patient with hemophilia A treated with DDAVP. *N Engl J Med* 1988; 318:121.

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 factor VIII concentrate but some nine days post-op, a third dose of DDAVP was given and a few hours later 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 that myocardial infarction is not surprising in a person of this age with claudication.)

McLeod BC Myocardial infarction in a blood donor after administration of desmopressin. *Lancet* 1990; 336:1137-1138.

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 Haemost* 1994; 71:154-155.

DDAVP came to be used in surgical procedures associated with heavy blood loss, especially during the 1980's when fear of AIDS transmission was maximal. Use of DDAVP greatly reduced blood loss but reports of myocardial infarcts and other thrombotic events frightened clinicians. The affected patients usually 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.

Levi M, Cromheecke ME, de Jonge E, Prins MH, de Mol BJ, Briet E, Buller Hr. Pharmacological strategies to decrease excessive blood loss in cardiac surgery: a meta-analysis of clinical relevant endpoints. *Lancet* 1999; 354:1940-47.

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

## Plasma fractions

See also "cross-transfusion experiments" early in the bibliography.

Biggs R, Matthews JM. The treatment of haemorrhage in von Willebrand's disease and the blood level of factor VIII (AHG). *Br J Haematol* 1963; 6: 203-14

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 improved, transiently. The authors concluded that correction of FVIII levels, but not correction of BTs, was important for hemostasis.

Borchgrevink CF, Egeberg O, Godal HC, Hjort PF. The effect of plasma and Cohn's fraction I on the Duke and Ivy bleeding times in von Willebrand's disease. *Acta Med Scand* 1963; 173:235-242.

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. (Differences in response may have correlated with VWD subtypes, not yet defined.)

Larrieu MJ, Caen JP, Meyer DO, Vainer H, Sultan Y, Bernard J. Congenital bleeding disorders with long bleeding time and normal platelet count. II. Von Willebrand's disease (Report of thirty-seven patients). *Am J Med* 1968; 45:354-372

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..."

Chediak JR, Telfer MC, Green D. Platelet function and immunologic parameters in von Willebrand's disease following cryoprecipitate and factor VIII concentrate infusion. *Am J Med* 1977; 62: 369-376.

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 than FVIII or VWF:Ag. 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.

Nilsson IM, Bergentz SE, Larsson SA. Surgery in von Willebrand's disease. *Ann Surg* 1979; 190: 746-752.

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 7<sup>th</sup> 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.

McLeod BC, McKenna R, Sassetti RJ. Treatment of von Willebrand's disease and hypofibrinogenemia with single donor cryoprecipitate from plasma exchange donation. *Am J Hematol* 1989 32:112-116.

Cryoprecipitate was made from the plasma of a single designated donor repeatedly plasmapheresed, as 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) per day. (See graph of post-op doses and levels.)

Pomper GJ, Rick ME, Epstein JS, Read EJ, Leitman SF. Management of severe VWD with cryoprecipitate collected by repeated apheresis of a single dedicated donor. *Transfusion* 2003; 43:1514-1521.

Cryoprecipitate was prepared from serial DDAVP-stimulated plasmapheresis of the father of a child with type 3 VWD. From infancy to age 12 years, the child was treated only with this product. A single infusion raising the plasma FVIII level to 50-100% was effective in stopping 97% of bleeding episodes.

Cesar JM, Garcia-Avello A, Monteagudo J, Espinosa JI, Lodos JC, Castillo R, Navarro JL. Von Willebrand factor availability in platelet concentrates stored for 5 days. *Am J Hematol* 1994; 45:109-111.

After storage for 5 days, platelet concentrates retained 82% of their baseline VWF:RCo content but the proportion of smaller molecular weight multimer increased. (Platelet transfusions are used in some situations and one might worry about the VWF stability of VWF at room temperature.)

#### FVIII-VWF concentrates

Fukui H, Nishino M, Terada S, Nichikubo T, Yoshioka A, Kinoshita S, Niinomi K, Yoshioka K. Hemostatic effect of a heat-treated factor VIII concentrate

(Haemate P®) in von Willebrand's disease. *Blut* 1988; 56:171-178.

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 detailed (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.

Berntorp E, Nilsson IM. Use of a high-purity factor VIII concentrate (Hemate P®) in von Willebrand's disease. *Vox Sang* 1989; 56:212-217.

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% post-operatively. (Multimer pictures after infusion illustrate the somewhat rapid disappearance of larger multimers.)

Lawrie AS, Harrison AL, Armstrong BR, Wilbourn RG, Dalton RC, Savidge GF. Comparison of the *in vitro* characteristics of von Willebrand factor in British and commercial factor VIII concentrates. *Br J Haematol* 1989; 73:100-104.

Multimer analysis and levels of VWF:RCo and VWF:CB were compared for FVIII concentrates available at the time. 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 from various concentrates.)

Rodeghiero F, Castaman G, Meyer D, Mannucci PM. Replacement therapy with virus-inactivated plasma concentrates in von Willebrand disease. *Vox Sang* 1991; 62:193-199, (review, 43 refs—recommended for figures)

Multimer patterns of VWF from 13 FVIII-VWF concentrates show that none had the largest multimers seen in normal plasma but some had higher molecular weight multimers than others.

Mannucci PM, Tenconi PM, Castaman G, Rodeghiero F. Comparison of four virus-inactivated plasma concentrates for treatment of severe von Willebrand disease: A cross-over randomized trial. *Blood* 1992; 79: 3130-3137.

An infusion of each of four concentrates, three containing both FVIII and VWF and one containing VWF but very little FVIII, were given to ten patients with severe VWD. The three FVIII-VWF concentrates raised levels of FVIII, VWF:RCo and VWF:Ag similarly; the VWF concentrate differed only in that levels of FVIII rose gradually over the first 24 hours. The Ivy BT was often somewhat shortened but rarely became normal.

Hanna WT, Bona RD, Zimmerman CE, Carta Ca, Hebert GZ, Rickles FR. The use of intermediate and high purity factor VIII products in the treatment of von Willebrand disease. *Thromb Haemost* 1994; 71:173-179.

After infusions of Koate HS® and Koate HP® (both are FVIII-VWF concentrates), levels of FVIII, VWF:Ag and VWF:RCo rose in patients with VWD. The Ivy BT was often somewhat improved but typically not corrected. Five patients underwent surgical operations. Three received an initial bolus dose of 25-40 FVIII U/kg at 24 hours pre-operatively, the others received immediate pre-operative boluses. Further concentrate was given by continuous infusion at a rate of two FVIII units per kg per hour. The rate of infusion was lower in the second week. Levels of VWF:RCo tended to be within the normal range, FVIII tended to be above the normal range, and VWF:Ag typically was more elevated than FVIII. (VWF:Ag levels reflect both low and high molecular weight VWF:Ag. The excellent graphs show the day-to-day variation in factor levels and also illustrate the wide disparity between super-high VWF:Ag, high FVIII and normal VWF:RCo levels during treatment.)

Mannucci PM, Lattuada A, Ruggeri ZM. Proteolysis of von Willebrand factor in therapeutic plasma concentrates. *Blood* 1994; 83:3018-3027.

Deficiency of the largest VWF multimers in concentrates made from pooled plasmapheresis plasma appeared to be due to increased proteolysis of VWF; proteolytic fragments were found. There was no evidence of fragmentation in cryoprecipitate made from single-donor plasma.

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."

Chang AC, Rick ME, Pierce LR, Weinstein MJ. Summary of a workshop on potency and dosage of von Willebrand factor concentrates. *Haemophilia* 1998; 4 (suppl 3), 1-6.

Clinical trials to date for pharmacokinetics, safety and efficacy in VWD of a few products from the USA and Europe are summarized. Humate-P® was said to contain about 2.5 units of VWF:RCo per unit of FVIII; Alphanate® contained about one unit of VWF:RCo per unit of FVIII; the French (LFB) VWF concentrate contained more than 10 units of VWF:RCo per unit of FVIII. HMW multimers were preserved fairly well in the LFB VWF concentrate and in Humate-P®. After infusions into patients of mixed VWD types, median half-lives of VWF:RCo were 11.3 hours with Humate-P® and 8.4 hours (VWD type 2A) and 10 hours (VWD type 3) with Alphanate. Measurements of VWF:RCo are "commonly regarded as the most appropriate present measurement". All the concentrates mentioned had been effective in clinical trials at the (*generous*) dosages used.

Nitu-Whalley IC, Griffioen A, Harrington C, Lee CA. Retrospective review of the management of elective surgery with desmopressin and clotting factor concentrates in patients with von Willebrand disease. *Am J Hematol* 2001; 66:280-4

During 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 more severe VWD.*)

Lillicrap D, Poon M-C, Walker I, Xie F, Schwartz BA. Efficacy and safety of the factor VIII/von Willebrand factor concentrate, Haemate-P/Humate-P: Ristocetin Cofactor unit dosing in patients with von Willebrand disease. *Thromb Haemost* 2002; 87:224-230.

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 minimum dosage needed for hemostatic efficacy was called a "contentious issue", because some patients are treated successfully with doses lower than 20 VWF:RCo U/kg. The minimum effective dose is "unresolved".

Favaloro EJ, Bukuva M, Martinelli T, Tzouroulis J, Duncan E, Welldon K, Collett M, Aumann H, Thom J, Gilmore F. A comparative multi-laboratory assessment of three factor VIII/von Willebrand factor concentrates. *Thromb Haemost* 2002; 87:466-76.

The old ("AHF High Purity") and the new ("Biostate") FVIII-VWF concentrates manufactured 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 appears

comparable to Humate-P in content of large multimers and proportion of functional VWF, and both were superior to AFH High Purity. (*A good figure depicts multimer distribution in the three concentrates.*)

Mannucci PM, Chediak J, Hanna W, Bymes J, Ledford M, Ewenstein BM, Retzius AD, Kapelan BA, Schwartz RS, Kessler C. Treatment of von Willebrand disease with a high-purity factor VIII/von Willebrand factor concentrate: a prospective, multicenter study. *Blood* 2002; 99:450-456.

A high-purity FVIII-VWF concentrate (Alphanate®) was used in 81 patients with VWD in an international study. In pharmacokinetic studies in patients with type 3 VWD, the post-infusion T<sub>1/2</sub> of FVIII was about 22 hours, of VWF:Ag was about 12 1/2 hours, and of VWF:RCo was about 7 hours. Acute bleeding episodes were controlled in 85% of instances with one or two infusions of 40 VWF:RCo units/kg in adults, 50 VWF:RCo U/kg in children. 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).

Mannucci PM. Venous thromboembolism in von Willebrand disease. *Thromb Haemost* 2002; 88:378-379.

Hemophilia centers around the world were surveyed to determine 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.

Makris M, Colvin B, Gupta V, Shields ML, Smith MP. Venous thrombosis following the use of intermediate purity FVIII concentrate to treat patients with von Willebrand's disease. *Thromb Haemost* 2002; 88:387-388.

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. In three, tests for factor V Leiden, prothrombin gene mutation and antiphospholipid antibodies were performed and were negative. The authors wonder whether a higher-than-normal level of FVIII induced by therapy might be dangerous.

Cox Gill J, Ewenstein BM, Thompson AR, Mueller-Veltens G, Schwartz BA. Successful treatment of urgent bleeding in von Willebrand disease with factor VIII/VWF concentrate Humate-P®: use of the ristocetin cofactor assay (VWF:RCo) to measure potency and to guide therapy. *Haemophilia* 2003; 9:688-695.

Dosage of Humate-P® was based on VWF:RCo international units (IU) in 33 patients with VWD who had 53 urgent bleeding episodes, the median initial dose was 67 IU of VWF:RCo per kg, the median daily dose was 74 IU, the median duration of treatment was two days. Efficacy was excellent or good in 98% of events. The highest doses were given to 11 patients with gastrointestinal bleeding (median 90 IU/kg) and two with intracranial bleeding (median 120 IU/kg). FVIII levels were recorded in eight patients treated for bleeding; the mean post-treatment peak was 113%, and two patients had peaks of 170 %.

Franchini M, Rossetti G, Tagliaferri A, Pattachini C, Pozzoli D, Lippi G, Manzato F, Bertuzzo D, Gandini G. Efficacy and safety of factor VIII/ von Willebrand factor concentrate (Haemate-P®) in preventing bleeding during surgery or invasive procedures in patients with von Willebrand's disease. *Haematologica* 2003; 88: 1279-1282.

Dosage Humate-P® was based on 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%; the highest 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.

Thompson AR, Gill JC, Ewenstein GM, Mueller-Veltens G, Schwarz BA. Successful treatment for patients with von Willebrand disease undergoing urgent surgery using factor VIII/VWF concentrate (Humate-P®). *Haemophilia* 2004; 10:42-51.

Humate-P® dosage was based on VWF:RCo international units. In 39 VWD patients of various 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 with minor ones. Recommended intervals were every 8-12 hours but actual intervals tended to be longer: the median number of infusions per day was 1.39 in the first 3 days, 1.21 on days 4-7 and 0.92 thereafter. Peak factor levels after the first infusion, measured in seven subjects; were a mean of 193% VWF:RCo and a mean of 122% FVIII. 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 that I give for surgery in hemophilia A during the same time period. Both dosage levels, for VWF and for hemophilia A, are generous and do not establish the minimum necessary dosage.)

Lethagen S, Carlsson M, Hillarp A. A comparative *in vitro* evaluation of six von Willebrand factor concentrates. *Haemophilia* 2004; 10:243-249.

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), Innobrand® (France, LFB) compared to normal plasma and to LFB's purified plasma VWF. HMW multimers were most nearly normal in Humate-P® and Innobrand®, followed by Koate DVI®, then 8Y. Levels of VWF:Ag were similar to those of VWF:RCo in Humate-P® and Innobrand® (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:

Concentrate	Units of VWF:RCo per unit of VWF:Ag	Units of FVIII per unit of VWF:RCo	% of HMW multimers > 7 <sup>th</sup>
Normal plasma	(presumed 1.0)	(presumed 1.0)	"100"
Purified VWF concentrate	0.72	0.02	82
Humate-P	0.91	0.50	91
Innobrand	0.89	0.40	100
Koate DVI	0.48	0.85	61
8Y	0.29	1.23	52
Immunate	0.15	6.00	15

Budde U, Metzner HG, Muller HG. Comparative analysis and classification of von Willebrand factor/ factor VIII concentrates: impact on treatment of patients with von Willebrand disease. *Semin Thromb Hemost* 2006; 32:626-635.

Federici AB, Castaman G, Franchini M, Morfini M, Zanone E, Coppola A, Tagliaferri A, Boeri E, Mazzuconi MC, Rosetti G, Mannucci PM. Clinical use of Haemate P in inherited von Willebrand disease: a cohort study on 100 Italian patients. *Haematologica* 2007; 92:944-951.

In a retrospective survey, Humate P had been given to 100 patients (77 with type 2 or 3 VWD) for 280 hemorrhages in a median dose of 72 IU/kg/day (range 27-135) with 95% good to excellent responses, for 73 surgical procedures in a median dose of 80 IU/kg/day (range 27-146) with 97% good to excellent responses, and for prophylaxis in 12 patients in a median dose of 72 IU/kg/day (range 24-96) with a 100% good to excellent response. In surgical cases, 18 patients also received tranexamic acid, one also received DDAVP and two received heparin. (I wish they had correlated dosage and response in their 37 patients with type 3 VWD.)

Shortt J, Dunkley S, Rickard K, Baker R, Street A. Efficacy and safety of a high purity, double virus inactivated factor VIII/ von Willebrand concentrate (Biostat®) in patients with von Willebrand disorder requiring invasive or surgical procedures. *Haemophilia* 2007; 13:144-146.

Biostat®, made in Australia, is a plasma-derived FVIII-VWF concentrate with excellent retention of HMW multimers. In a retrospective review, 43 patients with VWD (26 type 1, 12 type 2, five type 3) had undergone 34 minor and 24 major surgical procedures using the product. Hemostasis was rated as good to excellent in all instances; it was a little less likely to be rated as excellent in type 3 VWD. The mean pre-operative dose for major surgery was 29 IU FVIII/kg, the mean duration of treatment was five days and the mean daily post-op dose was 29 IU FVIII/kg. Pre-operative doses for minor surgery were similar. In this survey, dosage was measured in FVIII units, not VWF:RCo units. In view of the slightly lesser response in type 3 VWD, one might want to be more generous in such patients.)

Lethagen S, Kryle PA, Castaman G, Haertel S, Mannucci PM. Von Willebrand factor/ factor VIII concentrates (Haemate P®) dosing based on pharmacokinetics: a prospective multicenter trial in elective surgery. *J Thromb Haemost* 2007; 5:1420-1430.

In 28 subjects with various types of VWD, a pharmacokinetic study was performed in advance of elective surgery, with a median dose of 79 IU VWF:RCo/kg. The mean *in vivo* recovery was 1.7 IU/dL for type 1 VWD (n=10), 2.0 IU/dL for type 2A (n=10) and 2.5 IU/dL for type 3 (n=8). The median terminal half-life of VWF:RCo was 22.1 hours for type 1 VWD, 15.1 hours for type 2A and 8.8 hours for type 3. For surgical operations, the median loading dose was 62.4 IU VWF:RCo/kg, but varied widely. Surgical hemostasis was excellent. The striking difference in recovery and half-life among types of VWD may help in planning post-operative treatment.

Franchini M, Targher G, Lippi G. Prophylaxis in von Willebrand disease. *Ann Hematol* 2007; 86:699-704. A review of reports of short-term and long-term prophylaxis.

Hubbard AR, Heath AB. Standardization of factor VIII and von Willebrand factor in plasma: calibration of the WHO 5th International Standard (02-150). *J Thromb Haemost* 2004; 2:1380-1384.

Calibration of this recent standard for FVIII, VWF:Ag, VWF:RCo and VWF:CB was achieved with the collaboration of 37 laboratories around the world, which compared the proposed standard to fresh normal plasma and to the previous standard.

### Purified VWF Concentrates

Goudemand J, Mazurier C, Marey A, Caron C, Coupez B, Mizon P, Goudemand M. Clinical and biological evaluation in von Willebrand's disease of a von Willebrand factor concentrate with low factor VIII activity. *Br J Haematol* 1992; 80:214-221.

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.

Goudemand J, Negrier C, Ounnoughene N, Sultan Y. Clinical management of patients with von Willebrand's disease with a VHP VWF concentrate: the

French experience. *Haemophilia* 1998; 4:48-52.

Solvent-detergent treated plasma-derived VWF concentrate, with very little FVIII, was 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, Roussel-Robert V, Goudemand J, Lee CA, Scharer I, Rothschild C, Berntorp E, Henriot C, Tellier Z, Bridey F, Mannucci PM. Treatment of severe von Willebrand disease with a high-purity von Willebrand factor concentrate (Wilfactin®): a prospective study of 50 patients. *J Thromb Haemost* 2007; 5:1115-1124.

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 given 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 in multiply-treated surgical patients, from endogenous production, 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. No evidence is offered about the necessary minimum dose.*

Turecek P, Gritsch H, Pichler L, Auer W, Fischer B, Mitterer A, Mundt W, Schlokot U, Dörner F, Brinkman HJM, van Mourik JA, Schwarz HP. In vivo characterization of recombinant von Willebrand factor in dogs with von Willebrand disease. *Blood* 1997; 90:3555-3567.

**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.**