von Willebrand's disease in the year 2003: towards the complete identification of gene defects for correct diagnosis and treatment

GIANCARLO CASTAMAN, AUGUSTO B. FEDERICI, FRANCESCO RODEGHIERO, PIER MANNUCCIO MANNUCCI

Background. von Willebrand's disease (VWD) is an autosomally inherited bleeding disorder caused by a deficiency or abnormality of von Willebrand factor (VWF). VWF is a multimeric adhesive protein which plays an important role in primary hemostasis by promoting platelet adhesion to the subendothelium at sites of vascular injury and platelet-platelet interactions in high shear-rate conditions. It is also the carrier of factor VIII (FVIII), thus indirectly contributing to the coagulation process. VWD has a prevalence of about 1% in the general population, but the figure for clinically relevant cases is lower (about 100/million inhabitants). Bleeding manifestations are heterogeneous: mucosal bleeding is typical of all VWD cases but hemarthrosis and hematomas may also be present when FVIII levels are low.

Information sources. Most cases appear to have a partial quantitative deficiency of VWF (type 1 VWD) with variable bleeding tendency, whereas qualitative variants (type 2 VWD), due to a dysfunctional VWF, are clinically more homogeneous. Type 3 VWD is rare and the patients have a moderate to severe bleeding diathesis because of the virtual absence of VWF, and a recessive pattern of inheritance. The diagnosis of VWD, especially type I, may be difficult, because the laboratory phenotype is highly heterogeneous and is confounded by the fact that factors outside the VWF gene (e.g., blood group) influence VWF levels. An array of tests is usually required to characterize the VWD types of the disorder and establish the best treatment modality.

Conclusions. The aim of treatment is to correct the dual defect of hemostasis, i.e. abnormal coagulation expressed by low levels of FVIII and abnormal platelet adhesion expressed by the prolonged bleeding time (BT). Desmopressin (DDAVP) is the treatment of choice for type 1 VWD because it corrects the FVIII/VWF levels and the prolonged BT in the majority of cases. In type 3 and in severe forms of type 1 and 2 VWD, DDAVP is not effective and for these patients plasma virally-inactivated concentrates containing FVIII and VWF are the mainstay of treatment. These concentrates are clinically effective and safe, although they do not always correct the BT.

Key words: von Willebrand factor, congenital von Willebrand's disease, genetic and molecular diagnosis, desmopressin, factor VIII/von Willebrand factor concentrates.

Haematologica 2003;88:94-108
http://www.haematologica.org/2003_01/88094.htm

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From the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, IRCCS Maggiore Hospital and University of Milan, (ABF, PMM), Department of Hematology and Hemophilia and Thrombosis Center San Bortolo Hospital, Vicenza, (FR, GC), Italy.

Correspondence: Augusto B. Federici, MD, Angelo Bianchi Bonomi Hemophilia Thrombosis Center, Department of Internal Medicine IRCCS Maggiore Hospital and University of Milan, via Pace 9, Milan, Italy. E-mail: augusto.federici@unimi.it

When, in 1926, Erik von Willebrand described a novel bleeding disorder in a large family from Foglo on the islands of Aland in the Gulf of Bothnia, he provided an impressive and exhaustive description of the disorder's clinical and genetic features. Unlike hemophilia, the epitome of inherited bleeding disorders, both sexes were affected, and mucosal bleeding was the dominant symptom. A prolonged bleeding time (BT) with a normal platelet count was the most important laboratory abnormality and a functional disorder of the platelets associated with a systemic lesion of the vessel wall was suggested as a possible cause of the disorder. However, he called the disease *hereditary pseudohemophilia*. To further complicate the issue, some authors subsequently called the disorder *vascular hemophilia*.

Only in the 1950s, was it demonstrated that the prolonged BT in these patients was associated with reduced factor VIII (FVIII), but we had to wait until the 1970s to clarify that the deficiency of a new factor, called von Willebrand factor and different from FVIII, was actually responsible for the disease. Surprisingly, the reduction of this factor caused low FVIII, pointing to the close relationships between the two proteins. In the 1980s, the cloning of the VWF gene set the basis for unraveling the molecular causes of the disorder.

The history of von Willebrand's disease (VWD) has been the subject of recent reviews.[1] In this article we discuss the progress and the problems of diagnosis and treatment of VWD today, in the year 2003, 77 years after the original description by Erik von Willebrand.

The von Willebrand factor

Von Willebrand's disease is the most frequent inherited bleeding disorder and is due to a deficiency and/or abnormality of von Willebrand factor (VWF). VWF is synthesized by endothelial cells and megakaryocytes. The gene coding for VWF has been cloned and located at chromosome 12p13.2. It is a large gene composed of about 178 kilobases and containing 52 exons. A non-coding, partial, highly homologous pseudogene has been identified in chromosome 22.[2] The pseudogene spans the gene sequence from exon 23 to 34.[2]

The primary product of the VWF gene is a 2,813 amino acid protein made of a signal peptide of 22 amino acids (also called a pre-peptide), a large pro-peptide of 741 amino acids and a mature VWF molecule containing 2,050 amino acids. In keeping with a recently proposed nomenclature,[3] numbering starts from the first amino acid of the signal peptide, so 764 is the first amino acid of the mature protein. Different protein regions, corresponding to four types of repeated domains (D1, D2, D', D3, A1, A2, A3, D4, B, C1, C2) of cDNA, are responsible for the different binding functions of the molecule (Fig-

ure 1). Mature VWF is the result of ordered intracellular processing, leading to the storage and/or secretion of a heterogeneous array of multimeric multidomain glycoproteins, collectively referred to as VWF.

Purified VWF visualized by electron microscopy appears either as a filamentous structure with a diameter of 2-3 nm and a length of up to 1300 nm, close to the diameter of platelets, or as a loosely coiled molecule with a diameter of 2-300 nm.[4] The building block of VWF multimers is a dimer made up of two single-chain pro-VWF molecules, joined through disulphide bonds within their C-terminal region. This reaction occurs after cleavage of the signal peptide and the subsequent translocation and glycosylation of the precursor molecules into the endoplasmic reticulum. The pro-VWF dimers are then transported to the Golgi apparatus where, after further post-translational modifications including processing of high mannose oligosaccharides, they are polymerized into very large molecules up to a molecular weight of $20,000 \times 10^3$ through disulphide bonds connecting the two N-terminal ends of each dimer. After polymerization, pro-VWF multimers move to the trans-Golgi network where the VWF pro-peptide (also called VWF: AgII) is cleaved off by a paired amino acid-cleaving enzyme (PACE or furin) and remains, at least within the cell, non-covalently associated with VWF. VWF pro-peptide is secreted into the circulation together with native VWF and can be measured in plasma with specific antibodies.[5]

VWF is secreted from the cell along a constitutive and a regulated pathway. The latter is used for rapid stimuli-induced release (e.g. by desmopressin through its binding on the vasopressin V2 receptor of endothelial cells) from specialized storage organelles of endothelial cells known as Weibel-Palade bodies. Only Weibel-Palade bodies or α-granules in platelets contain fully processed and functional VWF with *unusually large* multimers, which are usually not found in circulation. Indeed, a specific plasma protease acts on VWF multimers released from the cell, cleaving the VWF subunit at the bond between Tyr1605 and Met1606 (Tyr842 and Met843 of the mature subunit), reducing the size of plasma VWF and creating the full spectrum of circulating VWF species, ranging from the single dimer to about 20 dimers in each VWF multimer.[6]

Regulatory cis-acting elements in the immediate upstream promoter region and first exon of the VWF gene and more complex transcriptional regulation pathways have been identified, which are responsible for cell and vascular bed-specific expression of VWF.[7] Besides being found in endothelial cells, megakaryocytes and platelets, VWF is present in the subendothelial matrix, where it is bound, through specific regions in its A1 and A3 domains, to different types of collagen.

VWF has two major functions in hemostasis. First, it is essential for platelet-subendothelium adhesion and platelet-to-platelet interactions as well as platelet aggregation in vessels in which rapid blood flow results in elevated shear stress, a function partially explored *in vivo* by measuring the BT. Adhesion is promoted by the interaction of a region of the A1 domain of VWF with GpIbα on platelet membrane. It is thought that high shear stress activates the A1 domain of the collagen-bound VWF by stretching VWF multimers into their filamentous form. Furthermore GPIbα and VWF are also necessary for platelet-to-platelet interactions.[8] The interaction between GPIbα and VWF can be mimicked in platelet–rich plasma by addition of the antibiotic ristocetin, which promotes the binding of VWF to GPIbα of fresh or formalin-fixed platelets. The A1 domain has been crystallized in complex with the Fab fragment of the function-blocking antibodies NMC-4. The solved structure at 2.2 Angstrong resolution provides information not only on the VWF residues interacting with the antibody, but also on the possible location of the GPIbα binding site.[7,8] Aggregation of platelets within the growing hemostatic plug is promoted by the interaction with a second receptor on platelets, GPIIb-IIIa (or integrin $\alpha_{IIb}\beta_3$), once activated, binds to VWF and fibrinogen, recruiting more platelets into a stable plug. Both these binding activities of VWF are highly expressed in the largest VWF multimers.

Second, VWF is the specific carrier of factor VIII (FVIII) in plasma. VWF protects FVIII from proteolytic degradation, prolonging its half-life in circulation and efficiently localizing it at the site of vascular injury. Each VWF monomer has one binding domain, located in the first 272 amino acids of the mature subunit (D′ domain) which can bind one FVIII molecule, *in vivo*; however only 1-2 % of available monomers are occupied by FVIII.[9] This explains why high molecular weight multimers are not essential for the carrier function of FVIII, although one would expect molecules of the highest molecular weight to be most effective in localizing FVIII at the site of vascular injury. In any case any change in plasma VWF level is usually associated with a concordant change in FVIII plasma concentration.

Classification of von Willebrand's disease

For further understanding of the terminology currently used, Table 1 summarizes the factor VIII/VWF complex nomenclature, as recommended by the International Society of Thrombosis and Hemostasis. Basically, the revised classification of VWD identifies two major categories, characterized by quantitative (types 1 and 3) or qualitative (type 2) VWF defects (Table 2). A partial quantitative deficiency of VWF in plasma and/or platelets

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Table 1. Recommended nomenclature of factor VIII/von Willebrand factor complex.

Factor VIII	
Protein	VIII
Antigen	VIII:Ag
Function	VIII:C
Von Willebrand factor	
Mature Protein	VWF
Antigen	VWF:Ag
Ristocetin cofactor activity	VWF:RCo
Collagen binding capacity	VWF:CB
Factor VIII binding capacity	VWF:FVIIIB

Table 2. Classification of von Willebrand's disease (modified from Sadler).[10]

Quantitative deficiency of VWF
Type 1. Partial quantitative deficiency of VWF
Type 3. Virtually complete deficiency of VWF

Qualitative deficiency of VWF
Type 2. Qualitative deficiency of VWF

A) Type 2A. Qualitative variants with decreased platelet-dependent function associated with the absence of high-molecular-weight VWF multimers.

B) Type 2B. Qualitative variants with increased affinity for platelet GPIbα.

C) Type 2M. Qualitative variants with decreased platelet-dependent function not caused by the absence of high-molecular-weight VWF multimers.

D) Type 2N. Qualitative variants with markedly decreased affinity for factor VIII.

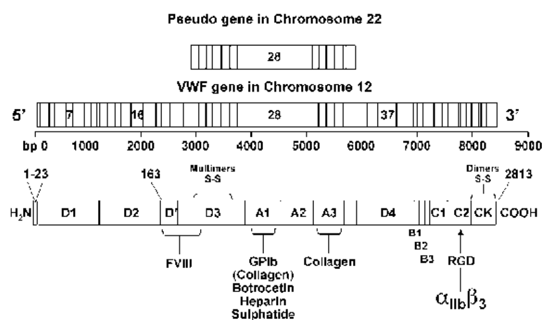

Figure 1. Schematic representation of the VWF gene located in chromosome 12 together with the pseudogene in chromosome 22. The main exons are indicated with the number of base pairs from 5′ to 3′ (above). DNA domain structure and pre-pro-VWF polypeptide: the pre-pro-VWF is indicated with amino acids numbered from the amino- (aa 1) to carboxy-terminal portions (aa 2813) of VWF. Note the important CK and D3 domains for formation of VWF dimers and multimers (below). The native mature subunit of VWF, after cleaving of the pre-pro VWF, is described with its functional domains: the VWF binding sites for factor VIII (D′ and D3), GPIbα, botrocetin, heparin, sulfatide, collagen (A1), collagen (A3) and the RGD sequence for binding to $\alpha_{IIb}\beta3$.

identifies type 1 VWD, whereas type 3 VWD is marked by the total absence or only trace amounts of VWF in plasma and platelets.[10] Type 1 is easily distinguished from type 3 by the milder VWF deficiency (usually in the range of 20-40 U/dL), the autosomal dominant inheritance pattern and the presence of milder bleeding symptoms. Four type 2 VWD subtypes have been identified, reflecting different pathophysiologic mechanisms.[10] Type 2A and 2B VWD are marked by the absence of high molecular weight VWF multimers in plasma; in type 2B, there is increased affinity for platelet GPIbα.

The identification of qualitatively abnormal variants with decreased platelet-dependent function and the presence of normal multimers on gel electrophoresis has led the addition of a new subtype, called 2M. If this definition is followed and more stringent criteria are applied to VWD diagnosis, many cases previously identified as type 1 VWD should now be classified as type 2M VWD because they are caused by single missense mutations affecting VWF function but not its multimeric structure and assembly (*see later*). Furthermore, type 2N (Normandy) also shows a full array of multimers since the defect lies in the N-terminal region of the VWF where the binding domain for factor VIII resides.[11] The subtype is phenotypically identified only by the FVIII/VWF binding test (*see later*).

Genetics and molecular biology of von Willebrand's disease

Cloning the VWF gene has allowed the identification of several suitable restriction fragment length polymorphisms (RFLP) which demonstrate the co-segregation of VWD phenotype with haplotype-specific RFLP patterns in family members of different kindred with VWD.[12]

Knowledge of the crucial segments of VWF involved in the interaction with GPIbα initially prompted the fruitful search for mutations in exon 28 of the VWF gene which encodes for the A1 and A2 domains of mature VWF as reported in Figure 1.[12] The search for mutations has been extended to additional VWF exons encoding for the other functional domains of VWF. The most frequent mutations reported in types 2A, 2B, 2M, 2N are listed in Table 3 according to the specific VWF domains and are currently updated in the web site organized on behalf of the Scientific Standardization Committee of the International Society of Thrombosis and Haemostasis (*www.shef.ac.uk/vwf*).

Most type 2A cases are due to missense muta-

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Table 3. List of the most frequent mutations in type 2A, 2B, 2M and 2N disease according to VWF domains.

Localization of VWF defects 1	*VWD types*	*VWF mutations associated with specific types*			
D2 domain	Type 2A (formerly IIC)	F404insNP	R436del6	N528S	G550R
		C623W	A625insG		
D' – D3 domains	Type 2N	R782W	G785E	E787K	C788R
		C788Y	T791M	Y795C	M800V
		R816W	R816Q	H817Q	R854Q
		R854W	C858F	D879N	Q1053H
		C1060R	C1225G		
D3 domain	Type 2M (formerly 1 Vicenza)	R1205H	Y1146C		
	Type 2A (formerly IIE)	C1143Y	C1173R		
A1 domain	Type 2B (formerly IIB)	P1266L	H1268D	C1272G	C1272R
		M1304insM	R1306Q	R1306L	R1306W
		R1308C	R1308P	I1309V	S1310F
		W1313C	V1314F	V1314L	V1316M
		P1337L	R1341L	R1341Q	R1341W
		L1460V	A1461V		
	Type 2M	G1324S	G1324A	E1359K	F1369I
		I1425F	Q1191del1	K1408delK	
	Type2M/2A	L1276P	R1374C	R1374H	C1458Y
		R1374R			
A2 domain	Type 2A (formerly IIA)	G1505E	G1505R	S1506L	F1514C
		K1518E	L1540P	S1543F	Q1556R
		L1562P	R1597G	R1597Q	R1597W
		V1604F	V1607D	V1609R	P1627H
		I1628T	G1629R	V1630F	E1638K
		L1639P	P1648S	L1657I	V1665E
		G1672R			
CK domain	Type 2A (formerly IID)	C2773R			

For an updated list of VWF mutations according to VWD types, the web site: www.shef.ac.uk/vwf can be checked.

tions in the A1 domain, with R1597W or Q or Y and S1506L accounting for about 60%.[12,13] Expression experiments have shown two possible mechanisms.[14] Group I mutations show impaired secretion of high molecular weight multimers, due to secondary defective intracellular transport. Group II mutations show normal synthesis and secretion of a VWF which is probably more susceptible to in vivo proteolysis (Table 3). The majority of type 2B cases are due to missense mutations in the A1 domain, about 90 % being caused by R1306W, R1308C, V1316M and R1341Q mutations.[12,13] A few heterogeneous mutations are responsible for type 2M cases and are also located within the A1 domain.[12,13] Therefore most mutations are expressed and the mutated recombinant VWF have been compared with others found within the same domain (Table 3).

A recurrent mutation in type 2M Vicenza has been recently reported in families from Europe (R1205H); it associated with a second nucleotide change (M740I) exclusively identified in some families from the Vicenza area.[15,16] Missense mutations in the FVIII-binding domain at the amino-terminal portion of VWF are responsible for type 2N.[17] The R854Q mutation is the most frequent and has found in about 2% of the Dutch population.[18] This mutation may cause symptoms only in homozygous or compound heterozygous states. Identification of the type 2N mutation, which is suspected in case of a marked reduction of FVIII in comparison to VWF and is confirmed by the FVIII/VWF binding test, is important for genetic counseling to exclude the state of carrier for hemophilia A.[11]

The inheritance of type 1 VWD is usually autosomal dominant, with variable phenotype and penetrance. Despite its high prevalence, the precise genetic cause of type 1 VWD is still elusive in most cases, especially those with a mild phenotype. Many type 1 VWD cases might be compound heterozygotes, producing an apparent dominant transmission[18] or alternatively, the mutated allele is negatively influenced by the effects of gene(s) outside the VWF gene and by other non-genetic factors contributing to the expression of a bleeding phenotype. In rare cases it is caused by frameshifts, nonsense mutations, or deletions that overlap those identified in type 3 VWD.[12] In a few cases with high penetrance, missense mutations have been described, for example of a cysteine in the D3 domain, resulting in a dominant negative mechanism.[19,20] In this case, mutant wild-type heterodimers are retained in the endoplasmic reticulum and only wild type homodimers are released into the circulation.[19]

The variable penetrance and severity of type 1 VWD may indeed be explained in some cases by the inheritance of two different VWD alleles.[18,21] Co-inheritance of R854Q mutation with a null mutation (for example, R2535X) increases the severity of bleeding within a given family.[18] In these families, simple heterozygotes show only minor bleeding symptoms.[18] However, in most cases with variable penetrance the genetic molecular background has not yet been investigated. In some of these families, linkage studies failed to establish a relation between the phenotype and a given VWF allele.[22] In these cases, a number of genetic and non-genetic factors are likely to contribute to the wide variability of the clinical and laboratory phenotype.[23]

About 60% of the variation in VWF plasma is due to genetic factors, with ABO group accounting for only about 30%.[24] In type O subjects the VWF level is 25-35% lower than in non-O individuals.[25] Thus, other unknown genetic factors may greatly influence VWF levels and, taken together with ABO

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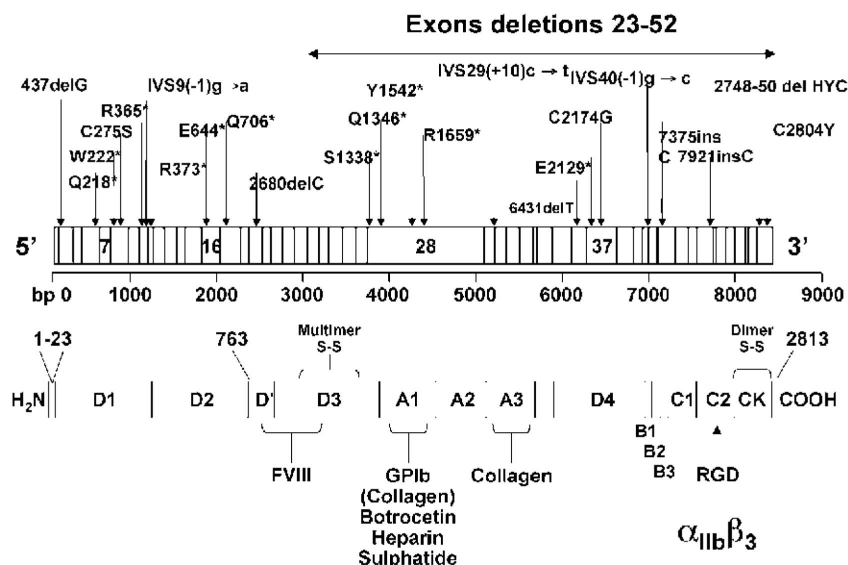

Figure 2. Distribution of the mutations found in the VWF coding region reported along with the homologous repeat domain structure. Note that no specific cluster for mutations is found in type 3 VWD as reported by Baronciani *et al.*[28]

Table 4. Prevalence of VWD: analysis of population studies.

Study	Methodology	Population	Prevalence
Rodeghiero *et al.*[34]	History + VWF:RCo Family study	Caucasian children	0.82 % (0.57 - 1.15)*
Rodeghiero *et al.*[35]	As above + VWF:Ag instead of VWF:RCo	As above	0.7 %
Miller *et al.*[36]	VWF:RCo	Adult blood donors	1.6 % (0.2 % bleeder)
Meriane *et al.*[37]	History + VWF:RCo Family study	Arabic - Turkish Adult students	1.23 %
Werner *et al.*[38]	History + VWF:RCo Family study	Caucasian - Black children	1.3 % (1.15 % Caucasian) 1.81 % Black)

**Prevalence taking into account the 90 % confidence interval for the lower limit of normal range.*

blood groups and environmental effects, help explain the wide variety and incomplete penetrance of type 1 VWD. Furthermore, at least one subset of type 1 may have a combination of genetic modifier mutations outside the VWF gene thus accounting for the failure of linkage studies. A possible example has been provided by a murine model of VWD, the RIIIS/J inbred mouse strain.[26] Using a positional cloning approach, the authors succeeded in identifying *Mvwf* as the unique allele for a previously known glycosyltransferase gene *Galgt2*. *Galgt2* is expressed primarily in the gut epithelium. Although its function in this tissue is unknown, gut specific expression is conserved in humans, suggesting an important role for the corresponding post-translational modification.

Since many aspects of type 1 VWD abnormalities are still not understood, a specific project entitled *Molecular and Clinical Markers for Diagnosis and Management of Type 1 VWD* was organized and sponsored by the European Union and the first results will be available within 2003.

In type 3 VWD, besides the mechanisms possibly shared with some type 1 cases (see above), partial or total gene deletions have been reported.[12] Notably, homozygosity for gene deletion may be associated with the appearance of allo-antibodies against VWF, which may render replacement therapy ineffective and stimulate anaphylactic reactions to treatment.[12] In general, mutations may be scattered over the entire gene, but some (e. g. 2680delC or Arg2535X) are particularly recurrent in Northern Europe.[12] The coding region of the VWF gene contains 11 CGA codons (Arg). CG-dinucleotides are hot spots for mutation and a C to T mutation will result in a stop codon. Stop codons, in either homozygosity or compound heterozygosity, have been reported in exons 9, 28, 32 and 45.[27] Gene defects of type 3 VWD patients from three different populations have now been studied,[28] but there was no founder effect and mutations were distributed throughout the entire VWF gene (Figure 2).

Prevalence and frequency of subtypes of von Willebrand's disease

VWD is the most frequent inherited bleeding disorder. Until the late 1980s, its prevalence was estimated from the number of patients registered at specialized centers. This approach gave an estimated prevalence ranging from 4 to 10 cases/100,000 inhabitants.[29-32] Bloom and Giddins in 1991, using the results obtained by a questionnaire sent to Hemophilia Centers worldwide,[31] gave an adjusted prevalence ranging from 0.37 to 23.9 cas-

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Table 5. Frequency of subtypes of von Willebrand's disease.

Authors	*Number of patients*	*Type 1 (%)*	*Type 2 (%)*	*Type 3 (%)*
Tuddenham[39]	134	75	19	6
Lenk *et al.*[40]	111	76	12	12
Nillson[31]	106 families	70	10	20
Hoyer *et al.*[41]	116	71	23	6
Awidi[42]	65	59	29.5	11.5
Berliner *et al.*[43]	60	62	9	29

Table 6. Incidence (%) of bleeding symptoms in patients with VWD and in normal subjects (adapted from Lak *et al.*;[48] Silwer;[49] Federici *et al.*).[50]

	Iranian VWD	*Italian VWD(*) (n = 1234)*			*Scandinavia*	
Symptoms	*Type 3 (n = 348)*	*Type 1 (n = 618)*	*Type 2 (n = 550)*	*Type 3 (n = 66)*	*VWD (n= 264)*	*Normals (n = 500)*
Epistaxis	77	61	63	66	62	5
Menorrhagia	69	32	32	56	60	25
Post-extraction bleeding	70	31	39	53	51	5
Hematomas	n. r.	13	14	33	49	12
Bleeding from minor wounds	n. r..	36	40	50	36	0.2
Gum bleeding	n. r..	31	35	56	35	7
Post-surgical bleeding	41	20	23	41	28	1
Post-partum bleeding	15	17	18	26	23	19
Gastrointestinal bleeding	20	5	8	20	14	1
Joint bleeding	37	3	4	45	8	0
Hematuria	1	2	5	12	7	1
Cerebral bleeding	n. r. .	1	2	9	n. r	0

n. r.: not reported; (*) Bleeding symptoms in Italian patients have been recently recalculated according to the updated results of the Italian Registry of VWD and are, therefore different from previously reported.[50]

Table 7. Clinical and laboratory parameters used for VWD diagnosis.

Patients at risk of VWD
- Clinical history: lifelong mucocutaneous and postoperative bleeding.
- Symptoms are sometimes present in other family members.
- Screening tests: prolonged bleeding time (maybe normal); normal platelet count; prolonged PTT (maybe normal).

Diagnosis and definition of VWD type
- VWF antigen [a]
- VWF: Ristocetin cofactor activity [b]
- Factor VIII [c]
- VWF multimeric structure on low resolution gels [e]

Diagnosis of VWD subtype
- Ristocetin-induced platelet agglutination (RIPA) [d]
- VWF multimeric structure on high resolution gels [e]
- Platelet VWF content [f]
- Factor VIII binding assay [g]

For the use of these tests see the diagnostic flow-chart reported in Figure 3 and also.[50]

es/100,000 population (Scandinavia). It is generally assumed that the number of people with symptomatic VWD requiring specific treatment, would be at least 100 per million.[33] Only a few studies have set out to estimate the prevalence of VWD by screening small populations using formal, standardized criteria. Table 4 summarizes the main characteristics of these studies.[34-38] In fact, the prevalence approaches 1% without ethnic differences. However, the large majority of cases diagnosed in population studies appear to have mild disease, and most of these subjects had not had any detailed hemostatic evaluation before.

It remains to be known whether these cases are due to mutations within the VWF gene or are the effect of an outside gene that influences the circulating level of VWF. Extensive haplotype studies or the demonstration, as in murine VWD,[26] of the effect of another gene are needed to clarify this issue. Table 5 summarizes the main reported series.[39-43] These estimates are obviously biased since many type 1 VWD cases without major symptoms are presumably not reported and almost all severe type 3 VWD are followed at specialized centers. Probably, however, the prevalence of type 3 is underestimated since the figures on this subtype were based mainly on mail surveys to leading hemophilia centers, with ill-defined inclusion criteria. In contrast, in population studies almost all cases were type 1,[35,39] providing further confirmation that mild cases are probably underestimated in series from specialized centers.

In the past the most frequent form of VWD was considered to be type I.[39-43] A recent retrospective study based on reappraisal of type 1 diagnoses after ten years (1992 versus 2002) in 316 VWD patients followed in a single Hemophilia Center, found that the numbers with types 3, 2A, 2B, 2N remained the same. Among the 215 (69%) type 1 VWD cases previously diagnosed, however, only 108 (34%) were confirmed as type 1. A total of 105 case (33%) showed abnormal VWF activity, as shown by a VWF:RCo/Ag ratio < 0.7.[44]

Clinical manifestations

The clinical expression of VWD is usually mild in type 1, the severity increasing in types 2 and 3. However, in some families the severity of bleeding manifestations varies, underlining the different molecular bases of the diverse phenotypes of this disorder, and its variable penetrance. In general, the severity of bleeding correlates with the degree of the reduction of FVIII:C, but not with the magnitude of BT prolongation or with the patient's ABO blood type.

Mucocutaneous bleeding (epistaxis, menorrha-

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Table 8A. Basic and discriminating laboratory assays for the diagnosis of VWD.

Test	*Pathophysiologic significance*	*Diagnostic significance*
Ristocetin Cofactor (VWF:RCo) using formalin-fixed platelets and fixed ristocetin concentration (1 mg/mL)[53]	VWF-GpIbα interaction as mediated by ristocetin *in vitro* (ristocetin at fixed concentration, normal platelets, patient plasma)	*Functional test*; most sensitive screening test
Immunologic assay with polyclonal antibody (VWF:Ag)[52]	Antigen concentration	Correlates with VWF:RCo in type 1; reduced ratio VWF:RCo/Ag suggests type 2 VWD
FVIII:C level (one-stage assay)	FVIII/VWF interaction	Not specific, but useful for patient management
Bleeding time (Ivy method)	Platelet-vessel wall VWF-mediated interaction	Not specific;correlates with platelet VWF content in type 1 VWD. Screens for qualitative platelet defects.
Ristocetin-induced platelet aggregation (RIPA)	Threshold of ristocetin concentration inducing patient platelet-rich plasma aggregation	Allows discrimination from type 2B, characterized by reduced threshold
Multimeric analysis	Multimeric composition of VWF	Presence of full range of multimers in types 1, 2M, 2N. Loss of high and intermediate multimers in types 2A and 2B.
Platelet VWF	Reflects also endothelial stores	Useful to predict responsiveness to desmopressin in type 1
Binding of FVIII to VWF	Interaction of normal FVIII with patient's plasma VWF	Allows discrimination from type 2N, characterized by low binding values

gia) is a typical manifestation of the disease and may even affect the quality of life. VWD may be highly prevalent in patients with isolated menorrhagia.[45] Women with VWD may require treatment with antifibrinolytics, iron supplementation or an estroprogestinic pill to control heavy menses. Bleeding after dental extraction is the most frequent post-operative bleeding manifestation. Since FVIII is usually only slightly reduced, manifestations of a severe coagulation defect (hemarthrosis, deep muscle hematoma) are rare in type 1 VWD and are mainly post-traumatic, whereas in type 3 the severity of bleeding may sometimes resemble that in patients with hemophilia. Bleeding after delivery is rare in type 1 VWD since FVIII/VWF levels tend to become normal at the end of pregnancy.[46] In a few cases, however, the FVIII/VWF levels do not become normal and these women need prophylaxis with DDAVP or factor VIII/VWF concen-

Table 8B. Other tests proposed for VWD diagnosis.

Test	Pathophysiologic significance	Diagnostic significance
Binding of VWF to collagen[60]	VWF-collagen interaction	Correlates with VWF :RCo in type 1 VWD; some collagen preparations more sensitive to high molecular weight multimers[61,62]
Closure time PFA-100[58,59]	Simulates primary hemostasis after injury to a small vessel	More sensitive than bleeding time in screening for VWD; not tested in bleeding subjects without specific diagnosis; specificity unknown; more data needed before recommendation for clinical laboratory
Monoclonal antibody-based ELISA[54]	Moab against an epitope of VWF involved in the interaction with GpIbα	Correlation with VWF:RCo not confirmed; not suggested instead of VWF:RCo[55]
Elisa-based *VWF:RCo*[56]	Measures interaction between VWF and captured rGpIbα fragment in the presence of ristocetin	Promising new test to use instead of VWF:RCo; more data needed

Moab: monoclonal antibodies.

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trates (*see later*) before delivery.[47] Women with type 2A, 2B and 3 VWD disease usually need replacement therapy post-partum to prevent immediate or late bleeding.[48] Post-operative bleeding may not occur even in more severely affected type 1 VWD patients, but in type 3 VWD prophylaxis is always required.

To date, only a few full descriptions of symptoms in VWD patients have been provided[48-50] and only one study took into account the differentiation according to the VWD types.[50] Table 6 shows the relative frequency of bleeding symptoms in three large series of patients with VWD diagnosed at specialized centers. In the Scandinavian experience, the percentage of patients with post-partum bleeding overlapped the percentage observed in normal females. It is striking that the distribution of different types of bleeding (apart from joint bleeding) is similar for the different subtypes. However, the severity of bleeding manifestations (for example menorrhagia or gastrointestinal bleeding) is more marked in type 3 VWD, often requiring replacement treatment. Guidelines for the diagnosis and therapy of VWD in Italy have recently been published[50] and some data will also be discussed in this review.

Diagnosis of von Willebrand disease

The spectrum of severity of VWD is wide, ranging from few, doubtful hemorrhagic symptoms to severe life-threatening bleeding episodes. This is due not only to the heterogeneous defects of the

VWF gene which may impair its hemostatic function, but also to the influence exerted by other genes (e.g., those for ABO blood groups). In addition, many acquired conditions, either physiologic (stress, pregnancy) or pathologic (inflammation), can induce fluctuations in VWF levels. Thus, the diagnosis of VWD, particularly type 1, may require several laboratory tests.

Screening tests. These tests are usually applied for patients with a suspected bleeding tendency and Table 7 summarizes the different steps for diagnosing VWD. The platelet count is usually normal, but mild thrombocytopenia may occur in patients with type 2B VWD. The *bleeding time* (BT) is usually prolonged, though it may be normal in patients with mild forms of VWD such as those with type 1 and normal platelet VWF content.[51] The *prothrombin time* (PT) is normal whereas the *partial thromboplastin time* (PTT) may be prolonged to a variable degree, depending on the plasma FVIII levels.

Diagnosis of VWD and identification of the type. The list of tests used to diagnose VWD and their pathophysiologic and diagnostic significance are reported in Table 8a.[52,53] *VWF antigen* (*VWF:Ag*) is unmeasurable in type 3 VWD, whereas it may be low in type 1 and low or normal in type 2. The assay for *ristocetin cofactor activity* (*VWF:RCo*) explores the interaction of VWF with platelet GPIbα and is still the standard method for measuring VWF activity. It is based on the property of the antibiotic ristocetin to agglutinate formalin-fixed normal platelets in the presence of VWF. Besides the original method,[53] new *ex vivo* methods have been proposed to measure the interactions between VWF and its platelet receptors in the presence of ristocetin, as reported in Table 8b.[54-56]

In patients with a normal VWF structure (type 1 VWD), VWF:RCo values are similar to VWF:Ag. Levels lower than VWF:Ag (VWF:RCo/Ag ratio < 0.7) are characteristic of type 2 VWD, as recently reported in the guidelines for diagnosis and treatment of VWD in Italy.[50] FVIII:C plasma levels are very low (1-5 %) in patients with type 3 VWD. In patients with type 1 or type 2 VWD, FVIII may be decreased to a variable extent but is sometimes normal.

Normal VWF is composed of a complex series of *multimers* with molecular weight ranging from 800 to 20,000 kDa, which can be analyzed by agarose gel electrophoresis. Low-resolution agarose gels distinguish VWF multimers, which are conventionally indicated as high, intermediate and low molecular weight. In types 1, 2M and 2N VWD all multimers are present, whereas in types 2A and 2B the high and intermediate multimers are missing.

Diagnosis of the subtype. For a correct diagnosis of patients with VWD and to establish their treatment, other assays are used to define specific subtypes. Ristocetin-induced platelet agglutination (RIPA) is measured by mixing different concentrations of ristocetin and the patient's platelet rich-plasma (PRP) in an aggregometer. Results are expressed as the concentrations of ristocetin (mg/mL) able to induce 30% agglutination. Most VWD types and subtypes show a low response to ristocetin, but an important exception is type 2B VWD, in which there is hyperresponsiveness to ristocetin, due to a higher than normal affinity of VWF for platelet GPIbα.[57]

VWF *multimeric analysis* with high-resolution agarose gels identifies VWD subtypes 1 and 2 better. *Platelet VWF* plays an important role in primary hemostasis, since it can be released from α-granules directly to the site of vascular injury. On the basis of its measurement, type 1 VWD can be classified in three subtypes: type 1 *platelet normal*, with a normal content of functionally normal VWF; type 1 *platelet low*, with low concentrations of functionally normal VWF; type 1 *platelet discordant*, with normal concentrations of dysfunctional VWF.[51]

The *factor VIII binding assay* measures the affinity of VWF for FVIII. In this assay, anti-VWF antibody is coated on wells of a microtiter plate and test plasma is added to the wells. The factor VIII/WF complex from the plasma is bound by the antibody after which factor VIII is removed from the complex by a high ionic strength buffer. Excess recombinant FVIII (rFVIII) is then added and, after removal of unbound rFVIII, the VWF and the bound rFVIII are assayed. This assay allows type 2N VWD to be distinguished from mild to moderate hemophilia A.

Diagnostic approach. The diagnosis of VWD subtypes can be made by using the methods described above, in the flow-chart already proposed in the guidelines for diagnosis and treatment of VWD in Italy (Figure 3). Type 3 VWD can be diagnosed in cases of unmeasurable VWF:Ag. A proportionate reduction of both VWF:Ag and VWF:RCo with a RCo/Ag ratio > 0.7 suggests type 1 VWD. If the VWF:RCo/Ag ratio is < 0.7 type 2 disease is diagnosed. Type 2B VWD can be identified by an enhanced RIPA (< 0.8 mg/mL) while type 2A and 2M cause low RIPA (> 1.2 mg/mL). Multimeric analysis in plasma is necessary to distinguish between type 2A VWD (lack of the largest and intermediate multimers) and type 2M VWD (all the multimers present as in normal plasma). Type 2N VWD can be suspected in the case of discrepant values for factor VIII and VWF:Ag (ratio <1) and diagnosis should be confirmed by the specific test of VWF:factor VIII binding capacity (VWF:FVIIIB).

In type 1 VWD the ratio between factor VIII and VWF:Ag is always > 1 and the severity of type 1 VWD phenotype can usually be evaluated from platelet VWF measurements.[50]

Additional tests for VWD diagnosis include the

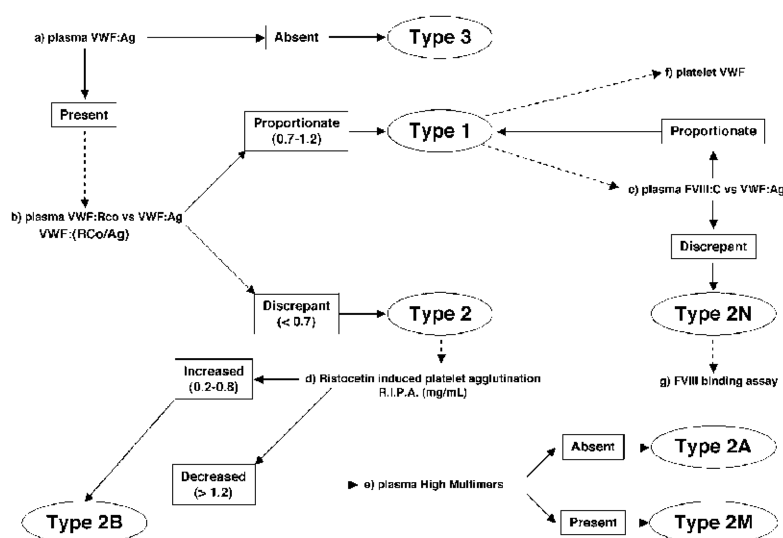

Figure 3. Diagnostic flow chart of different VWD types. Type 3 VWD can be diagnosed in case of unmeasurable VWF:Ag (a). A proportional reduction of both VWF:Ag and VWF:RCo with a RCo/Ag ratio > 0.7 suggests type 1 VWD (b). If the VWF:RCo/Ag ratio is < 0.7 type 2 is diagnosed. Type 2B VWD (d) can be identified in case of an enhanced RIPA (< 0.8 mg/mL) while type 2A and 2M cause low RIPA (> 1.2 mg/mL). Multimeric analysis in plasma (e) is necessary to distinguish between type 2A VWD (lack of the largest and intermediate multimers) and type 2M VWD (all the multimers present as in normal plasma). Type 2N VWD can be suspected in case of discrepant values for factor VIII (c) and VWF:Ag (ratio <1) and diagnosis should be confirmed by the specific test (g) of VWF:factor VIII binding capacity (VWF:FVIIIB). In type 1 VWD the ratio between factor VIII and VWF:Ag is always > 1;the severity of type 1 VWD phenotype can usually be evaluated from platelet VWF (f) measurements.[50] See also Tables 7 and 8A.

closure time (CT) and assays of VWF activity based on binding to collagen (*VWF:CB*). The CT can be evaluated with the Platelet Function Analyzer (PFA-100), which gives a rapid and simple measure of VWF-dependent platelet function at high shear stress. This system is sensitive and reproducible for VWD screening, even though the CT is normal in type 2N disease.[58,59] Assays are also available for VWF:CB and the ratio of VWF:CB to VWF:Ag appears to be useful for distinguishing types 1 and 2.[60-62] Neither assay has been well standardized yet and thus are not officially approved by the Scientific Standardization Committee on VWF of the International Society of Thrombosis and Haemostasis.

Management of patients with VWD

The goal of therapy in patients with VWD is to correct the dual defect of hemostasis, i.e. the abnormal platelet adhesion and the abnormal intrinsic coagulation pathway due to low FVIII levels. There are two treatments of choice, desmopressin or transfusional therapy with blood products. Other forms of treatment can be considered as adjunctive or alternatives to these.[63,64]

Desmopressin

Desmopressin (1-deamino-8-D-arginine vasopressin, DDAVP) is a synthetic analog of vasopressin originally designed for the treatment of diabetes insipidus. DDAVP raises FVIII and VWF plasma concentrations with no major side effects in healthy volunteers or patients with mild hemophilia and VWD.[65] Its mechanism of action has been recently investigated.[66] The first successful clinical trial with DDAVP was in 1977, its aim being to avoid the use of blood products in mild hemophilia and VWD patients who needed dental extractions and other surgical procedures.[67] Following these early observations, DDAVP has been widely used for the treatment of these diseases.[68] The obvious advantages is that DDAVP is relatively inexpensive and carries no risk of transmitting blood-borne viruses. DDAVP is usually infused intravenously over 30 minutes at a dose of 0.3 mg/Kg diluted in 50 mL saline. This increases plasma FVIII/VWF 3 to 5 times above the basal levels within 30-60 minutes and, in general, high FVIII/VWF concentrations last for 6 to 8 hours.

Since the responses in a given patient are consistent on different occasions,[69] a test dose of DDAVP at the time of diagnosis helps to establish the individual response patterns. Infusions can be repeated every 12 to 24 hours depending on the type and severity of the bleeding episode. However, most patients treated repeatedly with DDAVP become less responsive to therapy.[70] The drug is also available in concentrated forms for subcutaneous and intranasal administration, which can be convenient for home treatment.[71]

The protocol of the DDAVP infusion test with the clinical and laboratory parameters used to assess the biological response in each patient are summarized in Table 9; the definition of response to DDAVP is also reported together with the list of DDAVP products commercially available in Italy.[50]

Side effects of DDAVP are usually mild tachycar-

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dia, headache and flushing: these are attributed to the vasomotor effects of the drug and can often be attenuated by slowing the rate of infusion. Hyponatremia and volume overload due to the anti-diuretic effects of DDAVP are relatively rare. A few cases have been described, mostly in young children who received closely repeated infusions.[71] Though no thrombotic episodes have been reported in VWD patients treated with DDAVP, this drug should be used with caution in elderly patients with atherosclerotic disease, because a few cases of myocardial infarction and stroke have occurred in hemophiliacs and uremic patients given DDAVP.[73,74] DDAVP is most effective in patients with type 1 VWD, especially those who have normal VWF in storage sites (type 1, *platelet normal*).[51,75] In these patients FVIII, VWF and the BT are usually corrected within 30 minutes and remain normal for 6-8 hours. In other VWD subtypes, responsiveness to DDAVP varies. The response is poor and short-lasting in type 1, *platelet low*.[51] In type 2A VWD, FVIII levels are usually raised by DDAVP but the BT is shortened in only a minority of cases.[76] Desmopressin is contraindicated in type 2B VWD, because of the transient appearance of thrombocytopenia.[77] However, there have been reports on its clinical usefulness in some 2B cases.[78] In any case, the platelet count should be checked during test-infusion to detect any non-classical type 2B cases with thrombocytopenia occurring after infusion.[79] In type 2N relatively high levels of FVIII are obtained after DDAVP, but released FVIII circulates for a shorter time in plasma because the stabilizing effect of VWF is impaired.[80] Patients with type 3 VWD are usually unresponsive to DDAVP. However, a subgroup of patients with severe recessive VWD has been reported, characterized by normalization of FVIII without any changes of the abnormal BT.[81] Interestingly, all these patients share a unique mutation (C2362F) in compound heterozygosity or homozygosity.[20]

Other non-transfusional therapies for VWD

Two other non-transfusional therapies are used in the management of VWD: antifibrinolytic amino acids and estrogens. Antifibrinolytic amino acids are synthetic drugs that interfere with the lysis of newly formed clots by saturating the binding sites on plasminogen, thereby preventing its attachment to fibrin and making plasminogen unavailable within the forming clot. Epsilon xaminocaproic acid (50 mg/Kg four times a day) and tranexamic acid (15-25 mg/kg three times a day) are the most frequently used antifibrinolytic amino acids. Both can be administered orally, intravenously or topically and are useful alone or as adjuncts in the management of oral cavity bleeding, epistaxis, gastrointestinal bleeding and menorrhagia. Being drugs that inhibit the fibrinolytic system, they carry a potential risk of thrombosis in patients with an underlying prothrombotic state. They are also contraindicated in the management of urinary tract bleeding.

Estrogens raise plasma VWF levels, but the response is variable and unpredictable, so they are not widely used for therapeutic purposes. It is common clinical experience that the continued use of oral contraceptives is very useful in reducing the severity of menorrhagia in women with VWD, even in those with type 3, despite the fact that FVIII/VWF levels are not modified.

Transfusional therapies

Transfusional therapy with blood products containing FVIII/VWF is at the moment the treatment of choice for patients unresponsive to DDAVP.[63,64] Early studies indicated that cryoprecipitate every 12-24 hours normalized plasma FVIII levels, shortened the BT and stopped or prevented clinical bleeding in VWD. Based on these observations cryoprecipitate has been the mainstay of VWD therapy for many years. However, in 1992 the analysis of published reports pointed out that the BT is not always corrected following cryoprecipitate.[82]

Virucidal methods cannot be applied to cryoprecipitate, so it carries a small but definite risk of transmitting blood-borne infections. Therefore, virus-inactivated concentrates, originally developed for the treatment of hemophilia A, play an important role in the current management of VWD patients unresponsive to DDAVP. Concentrates obtained by immunoaffinity chromatography on monoclonal antibodies (FVIII >2,000 IU/mg) contain very small amounts of VWF and are therefore unsuitable for VWD management. A chromatography-purified concentrate particularly rich in VWF and with a very low content of FVIII has also been produced; it is called very-high purity VWF concentrate.[83] It was effective in a small cohort of type 3 VWD cases.[84] The very low content of FVIII in this concentrate sometimes makes it necessary, for the treatment of acute bleeding episodes and for emergency surgery, to infuse VWD patients also with a single first dose of purified FVIII concentrate to ensure immediate correction of the low FVIII levels.

No further use of FVIII concentrate is required later on, because the infused VWF triggers the endogenous synthesis of FVIII with normalization of FVIII levels after 6-8 hours. This might offer an example for the near future of a more specific therapeutic approach, as proposed by some French hematologists.[85] The dosages of concentrates recommended for the control of bleeding episodes are summarized in Table 11. The characteristics of products containing FVIII/VWF commercially available in Italy, partially modified from those reported in the Guidelines for diagnosis and therapy of VWD in Italy, are summarized in Table 10.[50] Since commercially available intermediate and high-purity FVIII/VWF concentrates contain large amounts of FVI-

Table 9. Products containing desmopressin commercially available in Italy.

Products (Company)	*Preparation*	*Volume/ ampoule*	*Number of ampoules/pack*	*Comments*
Emosint (*Kedrion*)	4 μg	0.5 mL	10	Concentrated ampoules can be administered also subcutaneously
	20 μg	1 mL	10	
	40 μg	1mL	10	
Minirin DDAVP (*Ferring/Valeas*)	4 μg	1 mL	10	

Infusion Test with Desmopressin

Infusion protocol	Administer in 30 minutes 0.3 μg/Kg of desmopressin in 50 mL of saline. The same dosage can also be administered subcutaneously.
Clinical and laboratory parameters	Factor VIII/VWF activities must be measured before and 0.5, 1, 2 and 4 hours after the administration of desmopressin; bleeding time must be measured at least before and after 2 hours. Check platelet count before and at least 2 hours after infusion
Definition of responsiveness	VWD patients should be considered responsive to desmopressin if *after 2 hours* they show at least 3-fold increases of baseline levels of FVIII:C and VWF:RCo, with levels of at least 30 U/dL and a bleeding time of 12 minutes or less, when prolonged.

Table 10. Characteristics of products containing FVIII/VWF commercially available in Italy.

Products (Company)	*Purification*	*Viral Inactivation*	*Specific activity* (U/mg prot)*	*VWF:RCo/ Ag° (Ratio)*	*VWF:RCo/ FVIII° (Ratio)*	*Other proteins*
AlphaNate (*Alpha Ther*)	Affinity Chromatography (Heparin)	Solv./Det. + 72 hrs at 80°C	>100	0.94	1.21	Albumin +
Emoclot D.I. (*Kedrion*)	Ion Exchange Chromatography	Solv./Det. + 30 min. a 100°C	≥ 80	0.61	1.16	Albumin -
Fanhdi (*Grifols*)	Affinity Chromatography (Heparin)	Solv./Det. + 72 hrs at 80°C	>100	0.83	1.48	Albumin +
Haemate P (*Aventis Behring*)	Multiple Precipitation	Pasteurization 10 hrs at 60°C	40±6	0.96	2.54	Albumin +
Immunate (*Baxter*)	Ion Exchange Chromatography	Det. + Vapour heat 10 h at 60°, 1h at 80°	100±50	0.47	1.10	Albumin +

**Specific activity measured as FVIII before adding albumin as stabilizer. ° VWF:RCo values are not available in the technical description of all concentrates: therefore only the mean values calculated by producers on different concentrate stocks could be reported.*

Table 11. Doses of factor VIII-VWF concentrates recommended in VWD patients unresponsive to DDAVP.

Type of bleeding	*Dose (IU/kg)*	*Number of Infusions*	*Objective*
Major surgery	50	Once a day or every other day	Maintain factor VIII > 50 U/dL for at least 7 days
Minor surgery	30	Once a day or every other day	factor VIII >30 U/dL for at least 5-7 days
Dental extractions	20-40	Single	factor VIII >30 U/dL for up to 6 hours
Spontaneous or post-traumatic bleeding	20-40	Single	

II and VWF, high post-infusion levels of these moieties are consistently obtained.[86-88] There is also a sustained rise in FVIII, higher than predicted from the doses infused, lasting up to 24 hours. This pattern is due to the stabilizing effect of exogenous VWF on endogenous FVIII, which is synthesized at a normal rate in these VWD patients.[89]

The accumulation of exogenous FVIII infused with the concentrates, together with that endogenously synthesized and stabilized by the infused VWF, causes very high FVIII levels when multiple infusions are given for severe bleeding episodes or to cover major surgery. There is some concern that sustained high levels of FVIII may increase the risk of post-operative deep vein thrombosis as recently suggested by several epidemiological studies.[90-92] Rare episodes of deep vein thrombosis have been reported in patients with VWD receiving repeated infusions of FVIII/VWF concentrates to maintain clinical hemostasis after surgery.[93-95]

These FVIII/VWF products are not always effective in correcting the BT[96] and there are probably various reasons for this. So far, no concentrate contains a completely functional VWF, as assessed *in vitro* by evaluating the multimeric pattern and using several functional assays, because VWF proteolysis occurs during purification due to the action of platelet and leukocyte proteases contaminating plasma used for fractionation.[97] It must borne in mind that the ideal exogenous FVIII/VWF concentrate with all its functional activities and all multimers can never correct the VWF defects in the subendothelium and in the platelets of VWD patients. On the other hand, in clinical practice, despite their limited and inconsistent effect on BT, FVIII/VWF concentrates are successfully used for the treatment of VWD patients unresponsive to DDAVP, especially for those with soft-tissue and post-operative bleeding.[82]

When the BT remains long and bleeding persists

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Table 12. Management of different types and subtypes of VWD.

	Treatment of choice	*Alternative and adjunctive therapy*
Type 1	Desmopressin	Antifibrinolytics, estrogens
Type 2A	Factor VIII/VWF concentrates	
Type 2B	Factor VIII/VWF concentrates	
Type 2M	Desmopressin	Factor VIII/VWF concentrates
Type 2N	Desmopressin	Factor VIII/VWF concentrates
Type 3	Factor VIII/VWF concentrate	Desmopressin, platelet concentrates
Type 3 with alloantibodies	Recombinant factor VIII	

despite replacement therapy, other therapeutic options are available. DDAVP given after cryoprecipitate further shortened or normalized BT in patients with type 3 VWD in whom cryoprecipitate had failed to correct the BT.[98] Platelet concentrates (given before or after cryoprecipitate, at doses of 4-5×10^{11} platelets) achieved similar effects, in terms of both BT correction and bleeding control, in patients unresponsive to cryoprecipitate alone.[99] These data emphasize the important role of platelet VWF in establishing and maintaining primary hemostasis. The therapeutic approaches according to VWD subtypes are summarized in Table 12.

Treatment of patients with allo-antibodies to VWF

For the rare patients with type 3 VWD who develop anti-VWF allo-antibodies after multiple transfusions, infusion of VWF concentrates not only is ineffective, but may even cause post-infusion anaphylaxis due to the formation of immune complexes.[100-102] These reactions may be life-threatening.[103,104] To overcome this drawback, a patient undergoing emergency abdominal surgery was treated with recombinant FVIII, because this product, which contains no VWF, could not cause anaphylactic reactions. In view of the very short half-life of FVIII without its VWF carrier, recombinant FVIII had to be administered by continuous i. v. infusion, at very large doses, to keep FVIII levels above 50 U/dL for 10 days after surgery.[104]

Conclusions

VWD is the most frequent inherited bleeding disorder. The prevalence of clinically relevant VWD has not been estimated directly in the population, but a figure around 100 cases/million people, similar to that of hemophilia A, is widely accepted. The phenotypic diagnosis is still the most accessible on account of the difficulties and costs of molecular diagnosis. However, molecular diagnosis can be useful to confirm specific VWF defects in VWD families. It is still not clear whether most mild type 1 VWD patients really have a mutation in the VWF locus. The results of a large multicenter European Study entitled *Molecular and Clinical Markers for Diagnosis and Management of type 1 VWD*, involving 150 VWD families, will soon be available and should provide insight into these issues. Despite its complex and heterogeneous nature, nowadays VWD can be efficiently and safely treated and mortality is virtually zero in Western countries.

Acknowledgments

A few data on diagnosis and management of VWD are from the Italian Registry of VWD sponsored by a grant from the Italian Ministry of Health. We wish to thank all the Members of the Italian Association of Hemophilia Centers who participated in this Registry and contributed to the preparation of the Guidelines for diagnosis and therapy of VWD in Italy. The style was kindly revised by Mrs. Judith Baggot.

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Pre-publication Report & Outcomes of Peer Review

This manuscript was solicited by the Editor-in-Chief and then peer-reviewed by two external referees. The final decision to accept this paper for publication was taken by Professor Mario Cazzola. Manuscript received April 11, 2002; accepted November 28, 2002.

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