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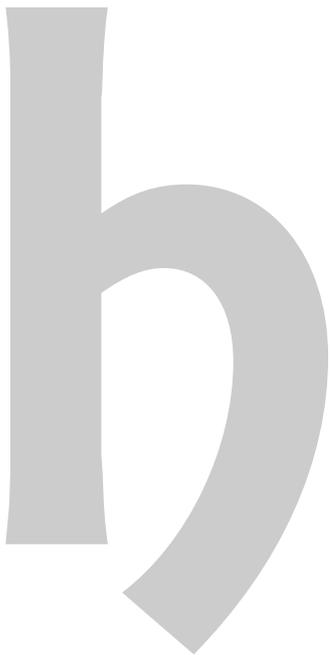
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**38th Congress of the
Italian Society
of Hematology
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von Willebrand's disease



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Journals (standard journal article,^{1,2} corporate author,³ no author given,⁴ journal supplement⁵):

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3. The Royal Marsden Hospital Bone-Marrow Transplantation Team. Failure of syngeneic bone-marrow graft without preconditioning in post-hepatitis marrow aplasia. *Lancet* 1977; 2:242-4.
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5. Karlsson S, Humphries RK, Gluzman Y, Nienhuis AW. Transfer of genes into hemopoietic cells using recombinant DNA viruses [abstract]. *Blood* 1984; 64(Suppl 1):58a.

Books and other monographs (personal authors,^{6,7} chapter in a book,⁸ published proceeding paper,⁹ abstract book,¹⁰ monograph in a series,¹¹ agency publication¹²):

6. Ferrata A, Storti E. *Le malattie del sangue*. 2nd ed. Milano: Vallardi; 1958.
7. Hillman RS, Finch CA. *Red cell manual*. 5th ed. Philadelphia: FA Davis; 1985.
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12. Ranofsky AL. *Surgical operation in short-stay hospitals: United States-1975*. Hyattsville, Maryland: National Center for Health Statistics; 1978. DHEW publication no. (PHS) 78-1785, (Vital and health statistics; series 13; no. 34).

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von Willebrand's disease

Proceedings of the Satellite Symposium
38th Congress of the Italian Society
of Hematology
Florence,
October 7-10, 2001

Epidemiology and diagnosis of von Willebrand's disease

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Epidemiology and diagnosis of von Willebrand's disease

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von Willebrand's disease (VWD) is an autosomally inherited bleeding disorder caused by a deficiency and/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. Moreover, it works as a carrier of factor VIII, thus indirectly contributing to the process of coagulation. The prevalence of VWD is about 1% in the general population, but that of clinically relevant cases is lower (about 100-200/million of inhabitants). Bleeding manifestations are heterogeneous and mainly linked to the degree of the reduction of factor VIII caused by low or abnormal VWF. Most cases 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 and account for about 20-30% of cases. Type 3 VWD is rare and the patients have a moderate to severe bleeding diathesis; the disease has a recessive pattern of inheritance and there is virtual absence of VWF. Heterozygous subjects are usually asymptomatic. The diagnosis of VWD may be difficult, especially that of type 1, since the laboratory phenotype of the disorder is very variable and heterogeneous and confounded by the influence on VWF levels by factors outside the VWF gene (e.g., blood group). An array of tests is usually required to characterize the several subtypes of the disorder in order to predict the best treatment modality.

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Key words: von Willebrand factor, von Willebrand's disease, inherited bleeding disorder

von Willebrand's disease



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Von Willebrand's disease (VWD) is the most frequent inherited bleeding disorder and is the result of a deficiency and/or abnormality of von Willebrand factor (VWF). VWF is an adhesive glycoprotein synthesized by megakaryocytes and endothelial cells. It circulates in plasma as an array of multimers, ranging from a dimer of 500 kD to molecular forms >10,000 kD (*for review see Ruggeri*¹). The gene coding for VWF has recently been cloned and localized in the short arm of chromosome.¹² 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 After removal of a signal peptide and of a prosequence of 741 amino acids and extensive intracellular processing including glycosylation, the secreted mature subunit contains 2,050 amino acids and displays a series of repetitive homologous domains with specific binding sequences for factor VIII, heparin, glycoprotein (GP) Ib, collagen and GPIIb/IIIa (*for review see Ruggeri*¹ and De Wit and Van Mourik³). Furthermore, a sequence present at -COOH termini (within the residues 1908-2050) and one at -NH₂ termini (within the residues 283-695) are responsible for dimerization and multimerization.¹

In platelets, VWF is stored in α -granules, whereas in endothelial cells it is mainly contained in the Weibel-Palade bodies as very large multimers released into the circulation after stimulation or secreted constitutively as dimers.³ Through specific domains, VWF interacts with GPIb on platelet membrane, leading to adhesion to the exposed subendothelium.¹ Potential binding sites in subendothelium are represented by collagen VI and heparin-like substances. After VWF/GPIb binding, platelets expose GPIIb/IIIa to which VWF binds

through an RGD sequence (amino acid residues 1744-1746) shared by other adhesive proteins. This binding makes adhesion irreversible, and contributes to platelet aggregation.¹ Furthermore, VWF is relevant to coagulation because of its ability to serve as a carrier of factor VIII through non-covalent bonds involving the -NH₂ termini, thus protecting it from proteolytic inactivation.^{1,3}

Table 1 summarizes the current nomenclature of factor VIII/VWF complexes, as recommended by the International Society on Thrombosis and Hemostasis.

Classification of von Willebrand's disease

Basically, the current revised classification of VWD identifies two major categories, characterized by quantitative (type 1 and 3) or qualitative (type 2) VWF defects. Furthermore, among type 2 four subtypes have been identified reflecting different pathophysiologic mechanisms (Table 2).⁴ Partial quantitative deficiency of VWF in plasma and/or platelets identifies type 1 VWD, whereas type 3 VWD is characterized by total absence or trace amounts of VWF in plasma and platelets.⁴ Until recently, the presence, although at a reduced concentration, of a fully array of multimers in the patient's plasma and/or platelets was considered sufficient evidence for type 1 VWD. Furthermore, type 1 is easily distinguished from type 3 by its milder VWF deficiency (usually in the range of 20-40%), the autosomal dominant inheritance pattern and the presence of milder bleeding symptoms. Classical type 2 A and B VWD are characterized by the absence of high molecular weight multimers of VWF in plasma. However, the identification of qualitatively abnormal variants with decreased platelet-dependent function and the

presence of normal multimers on gel electrophoresis has complicated the classification, requiring the addition of a new subtype, called 2M. Many, if not most type 1 cases, could be shifted to type 2M using more sophisticated functional and structural analyses capable of revealing single point mutations, affecting function but not multimeric structure and assembly. Furthermore, also type 2N (Normandy) 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.⁵ The subtype is phenotypically identified only by the FVIII-VWF binding test. Misclassification could also occur when a mutation for VWD type 2N is in compound heterozygosity with a type 1 or 3 mutation.⁶

Genetics and molecular biology of von Willebrand's disease

The cloning of 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 RFLPs pattern in family members of different kindreds with VWD (for review⁷). The knowledge of crucial segments of VWF involved in the interaction with GPIb initially prompted the fruitful search for mutations in exon 28 of the VWF gene which codes for the A1 and A2 domains of mature VWF (for review⁷). Most of the type 2A cases are due to missense mutations in the A1 domain. In particular R1597W or Q or Y and S1506L represent about 60% of cases.⁷ Expression experiments have demonstrated two possible mechanisms.⁸ 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

Table 1. Recommended nomenclature of factor VIII/von Willebrand factor (VWF) complexes.

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:FVIII-B

Table 2. Classification of von Willebrand's disease (modified from Sadler⁴).

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 GPIb
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

VWF which is probably more susceptible to *in vivo* proteolysis. The vast majority of type 2 B cases are due to missense mutations in the A1 domain. About 90% of cases are due to R1306W, R1308C, V1316M and R1341Q mutations.⁷ Usually type 2 A and B are autosomal dominant diseases with high penetrance and expressivity. Missense mutations in the FVIII-binding domain at NH2-termini of VWF are responsible for type 2N VWD (for review^{5,7}). The R854Q is the most frequent mutation observed and it has been found in about 2% of the Dutch population.⁶ The identification of type 2N mutation, which is suspected in the presence of a marked reduction of FVIII in comparison to VWF and is confirmed by the FVIII-VWF binding test, is important for genetic counselling to exclude the presence or carriage of hemophilia A.^{5,7} A few heterogeneous mutations are responsible for type 2M cases.⁷

The inheritance of type 1 VWD is usually autosomal dominant, with variable expressivity and penetrance. Despite its high prevalence, the precise genetic cause of type 1 VWD still remains elusive in most cases, especially in those with a mild phenotype. Many type 1 VWD cases might be compound heterozygotes producing an apparent dominant transmission⁶ or alternatively, the mutated allele might be negatively influenced by epistatic 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 with those identified in type 3 VWD.⁷ In a few cases displaying high penetrance and expressivity, missense mutations have been described, for example of a cysteine in the D3 domain, resulting in a dominant negative mechanism.^{8a,9} In this case, mutant-wild type heterodimers are retained in the endoplasmic reticulum and only wild type homodimers are released into circulation.^{8a} The variable penetrance and severity of type 1 VWD may indeed be explained in some cases by the inheritance of two different VWD alleles.^{6,10} It has been demonstrated that the coinheritance of R854Q mutation with a null mutation (for example, R2535X) increases the severity of bleeding within a given family.⁶ In these families, simple heterozygotes show only minor bleeding symptoms.⁶ However, the genetic molecular background of most cases showing variable penetrance and expressivity has not yet been investigated. In some of the families, linkage studies failed to establish a relation of the phenotype with a given VWF allele.¹¹ In these cases, a number of genetic and non-genetic factors are likely to con-

tribute to produce the wide variability in the clinical and laboratory phenotype.¹² About 60% of the variation in plasma VWF is due to genetic factors, with the ABO group accounting for only about 30%.¹³ In type-O subjects level of VWF is 25-35% lower than that in non-O individuals.¹⁴ Thus likely, other unknown genetic factors may greatly influence VWF levels and, taken together with ABO and environmental effects, explain the wide variation and incomplete penetrance of type 1 VWD. Furthermore, it is possible that there is a combination of genetic modifier mutations outside the VWF gene in at least a subset of type 1 disease, thus accounting for the failure of linkage studies. A possible example has been provided by a murine model of VWD, the RIIS/J inbred mouse strain.¹⁵

In addition to the mechanisms possibly shared with some type 1 cases (see above), partial or total gene deletions have been reported in type 3 VWD.⁷ Notably, homozygosity for the gene deletion may be associated with appearance of antibodies against VWF, which may render replacement therapy ineffective and stimulate anaphylactic reaction upon treatment.⁷ In general, mutations may be scattered over the entire gene; however, some mutations (e.g 2680delC or Arg2535X) are particularly recurrent in North Europe.⁷

Prevalence of von Willebrand's disease

A) Population studies. Only a few studies have tried to estimate the prevalence of VWD by screening small populations using formal, standardized criteria. The first study published in 1987 evaluated 1,218 schoolchildren aged 11-14 years in 4 small towns of the Veneto region in Northern Italy.¹⁶ A diagnosis of VWD was considered only for symptomatic children who had low VWF levels (VWF:RCo) and were members of a family with a convincing history of bleeding (probable VWD). A definite diagnosis was assigned if, in addition to these criteria, at least one other family member on the hemorrhagic side had a low VWF level. Age-adjusted separate ranges were adopted for O and non-O blood groups. Ten children (4 with probable and 6 with definite VWD) were classified as being affected (0.82%). This figure could range from 7 (0.57%) to 14 (1.15%) taking into account the 90% confidence interval for the lower limit of the normal range. The criteria for diagnosis were strictly conservative even though any bleeding symptoms except trivial were considered.

A subsequent study carried out on the same sample but using VWF:Ag instead of VWF:RCo as the screening test gave a slightly lower figure

showing that VWF:RCo is the more sensitive test.¹⁷ In 1993, Werner *et al.*¹⁸ published the results of a similar investigation carried out in 600 American adolescent schoolchildren aged 12-18 years. The criteria included at least one bleeding symptom, a family member with bleeding symptoms and low VWF. The overall prevalence was estimated to be 1.3%, with no racial difference (1.15% among Caucasians and 1.8% among Blacks). These data have been confirmed in two additional studies, not reported as full papers. Miller *et al.*¹⁹ in 1987 found a prevalence of VWD of 1.6% in adult blood donors from New York; however, the prevalence of symptomatic subjects with low VWF:RCo was 0.2%. In an additional study, Meriane *et al.*²⁰ investigated the prevalence of VWD in Arabic-Turkish adult subjects. The figure was 1.23%, again with no racial differences. Table 3 summarizes the main characteristics of these studies. The large majority of subjects picked up by population studies appear to have mild disease, and most of them had not required detailed hemostatic evaluation before. It remains unknown whether these cases are due to mutations within the VWF gene or are the effect of a gene outside the VWF gene influencing the circulating level of VWF. Only extensive haplotype studies or the demonstration, as in murine VWD,²¹ of the effect of another gene could definitely clarify this issue.

B) *Referral based.* In this case, estimates of the prevalence of VWD are based on the number of patients registered at a specialized center, divided by the total population served by that center. Using this approach, the prevalence has been estimated to range from 4 to 10 cases/100,000 inhabitants.²²⁻²⁴ For example, in 1982 Nillson, without any further specification on the methodology used, estimated that there were about 530 known cases (230 families) of VWD in Sweden (7/100,000 inhabitants), the same figure as for hemophilia in that country.²⁴ An additional approach to the estimation of VWD was tried more recently by Bloom and Giddins in 1991 using a mail questionnaire to Hemophilia Centers worldwide.²⁵ Underreporting was evident from some countries, notably North America. The authors gave an adjusted prevalence ranging from 0.37 to 23.9/100,000 (Scandinavia).

The major limitations of these estimations are that they rely on the assumption that all the potential patients have been identified, adequately examined and referred to the diagnostic center. However, a number of problems hamper the correct estimation of the prevalence of VWD based on referral. First of all, VWD has a variable penetrance

Table 3. Prevalence of VWD: analysis of population studies.

Study	Methodology	Population	Prevalence
Rodeghiero <i>et al.</i> ¹⁶	History + VWF:RCo Family study	Caucasian children	0.82% (0.57-1.15)*
Rodeghiero <i>et al.</i> ¹⁷	As above + VWF:Ag instead of VWF:RCo	As above	0.7%
Miller <i>et al.</i> ¹⁹	VWF:RCo	Adult blood donors	1.6% (0.2% bleeder)
Meriane <i>et al.</i> ²⁰	History + VWF:RCo Family study	Arabic-Turkish Adult students	1.23%
Werner <i>et al.</i> ¹⁸	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.

and expressivity and not infrequently patients may remain asymptomatic throughout their life. The severity of bleeding symptoms also varies within a given family.²⁶ Furthermore, as mentioned above, several variables may influence the level of circulating VWF.^{12,14,27} Finally, the diagnostic criteria are still far from being a definite validation, because of the difficulty in standardizing tests and the low sensitivity and specificity of laboratory tests. Reasonably, it could be assumed that the number of people with symptomatic VWD, requiring specific treatment, should be at least 100 per million, a widely quoted prevalence figure for clinically relevant cases.²⁸

Frequency of subtype of von Willebrand's disease

The characterization of VWD subtype usually requires the analysis of the multimeric pattern of plasma VWF for a broad distinction between types 1 and 2. This allows the selection of the proper treatment (desmopressin or FVIII/VWF concentrates) for most patients.²⁹ Specialized centers have usually been able to subclassify their patients in these ways. Table 4 summarizes the main series reported. These estimates are obviously biased since it is expected that many type 1 cases without major symptoms are not referred and that almost all severe type 3 are followed at a specialized center. Nevertheless, the prevalence of type 3 is probably also underestimated since the surveys on the prevalence of this subtype were based mainly on mail survey to leading hemophilia centers

Table 4. Frequency of subtypes of von Willebrand's disease.

Authors	Number of patients	Type 1 (%)	Type 2 (%)	Type 3 (%)
Tuddenham <i>et al.</i> ³⁰	134	75	19	6
Lenk <i>et al.</i> ³¹	111	76	12	12
Nilsson ²⁴	106 families	70	10	20
Hoyer <i>et al.</i> ³²	116	71	23	6
Awidi ³³	65	59	29.5	11.5
Berliner <i>et al.</i> ³⁴	60	62	9	29
Bloom & Giddins ²⁵	16,664*	55	8	3.5

*Information lacking for 33%.

Table 5. Incidence (%) of bleeding symptoms in patients with von Willebrand's disease and in normal subjects (adapted from Silwer⁴¹ and Federici & Mannucci⁴⁰).

Symptoms	Italian VWD (n = 1286)			Scandinavia	
	Type 1 (n=944)	Type 2 (n=268)	Type 3 (n=74)	VWD (n=264)	Normal (n=500)
Epistaxis	56	63	74	62	5
Menorrhagia	31	32	32	60	25
Post-extraction bleeding	31	39	53	51	5
Hematomas	14	19	31	49	12
Bleeding from minor wounds	36	40	50	36	0.2
Gum bleeding	30	37	48	35	7
Post-surgical bleeding	20	23	41	28	1
Post-partum bleeding	17	18	26	23	19
Gastrointestinal bleeding	5	11	18	14	1
Joint bleeding	2	5	42	8	0
Hematuria	2	4	11	7	1
Cerebral bleeding	0.5	0.3	8	N.R.	0

with ill-defined inclusion criteria and for the purpose of the feasibility of a study of atherosclerosis in VWD.^{35,36} In contrast to the above mentioned percentages, almost all cases in population studies had type 1 disease,^{16,18} further confirming that mild cases are probably underestimated in series from specialized centers.

Clinical manifestations

Clinical expression of VWD is usually mild in type 1 disease, increasing in severity in type 2 and type 3. However, in some families a variable severity of bleeding manifestations is evident, underlining the different molecular bases responsible for the diverse phenotypes of the 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 ABO blood type of the patient. Mucocuta-

neous bleeding (epistaxis, menorrhagia) is a typical, prominent manifestation of the disease and may affect quality of life. VWD may be highly prevalent in patients with isolated menorrhagia.³⁷ Females with VWD may require treatment with antifibrinolytics, iron supplementation or an estrogenic pill to control heavy menses. Bleeding after dental extraction is the most frequent post-operative bleeding manifestation. Since FVIII:C is usually only mildly reduced, manifestations of a severe coagulation defect (hemarthrosis, deep muscle hematoma) are rarely observed in type 1 VWD and are mainly post-traumatic, whereas in type 3 the severity of bleeding may sometimes be similar to that of hemophilia. Bleeding after parturition is rarely observed in type 1 disease since FVIII/VWF levels tend to correct towards the end of pregnancy in mild type 1 cases.³⁸ However, FVIII/VWF levels fail to normalize in a few cases and these need antihemorrhagic prophylaxis for parturition.³⁹ Women with type 2A and B and type 3 disease usually need replacement therapy post-partum to prevent immediate or late bleeding. Post-operative bleeding may not occur even in more severely affected type 1 patients, except following from procedures which involve richly vascular tissues or organs (e.g., tonsillectomy, thyroidectomy), whereas prophylactic treatment is always required in patients with type 3 disease.

To date, only two descriptive reports of symptoms in VWD have been provided^{40,41} and only in one was a differentiation according to subtype taken into account.⁴⁰ Table 5 shows the relative frequency of bleeding symptoms in these two large series of patients with VWD diagnosed at specialized centers. Notably, in the Scandinavian experience, the percentage with post-partum bleeding overlaps that observed in normal females. It is striking that the distribution of different types of bleeding (apart from joint bleeding) is similar among the different subtypes. It should, however, be borne in mind that the severity of bleeding manifestations (for example menorrhagia or gastrointestinal bleeding) is more prominent in type 3 VWD, often requiring substitutive treatment.⁴²

Diagnosis of von Willebrand's disease

VWD has a wide spectrum of severity ranging from few doubtful hemorrhagic symptoms to severe lifethreatening bleeding episodes. This variability is due not only to the many different molecular defects of the VWF gene which may impair its hemostatic function, but also the influences exerted by other genes (e.g., those specifying the ABO

Table 6. Basic and discriminating laboratory assays for the diagnosis of von Willebrand's disease.

Test	Pathophysiological significance	Diagnostic significance
Ristocetin cofactor (VWF:RCo) using formalin-fixed platelets and fixed ristocetin concentration (1 mg/mL)	VWF-Gp Ib interaction as mediated by ristocetin <i>in vitro</i> (ristocetin, normal platelets, patient's plasma)	"Functional test"; most sensitive screening test
Immunologic assay with polyclonal antibody (VWF:Ag)	Antigen concentration	Correlates with VWF:RCo in type 1; reduced VWF:RCo/VWF:Ag (< 0.7) suggests type 2 VWD; level < 3 U/dL suggests type 3 VWD
FVIII:C level (one-stage assay)	FVIII-WWF interaction	Not specific, but useful for patient management; disproportionately reduced compared to VWF in type 2N VWD
Bleeding time (Ivy method)	Platelet-vessel wall VWF-mediated interaction	Not specific; correlates with platelet VWF content in type 1 VWD
Ristocetin-induced platelet aggregation (RIPA) using patient platelets	Threshold ristocetin concentration inducing patient's platelet-rich plasma aggregation	Allows the discrimination of type 2B, characterized by reduced threshold; absent in type 3 at every ristocetin concentration
Multimeric analysis (low resolution gel)	Multimeric composition of VWF	Presence of full range of multimers in type 1; high molecular weight multimers absent in type 2 A and B; multimers absent in type 3
Platelet VWF	Reflects endothelial stores	Useful to predict responsiveness to desmopressin in type 1
Binding of VIII:C to VWF	Interaction of normal FVIII with patient plasma VWF	Allows the identification of type 2N, characterized by low binding values

blood group). Furthermore, many acquired conditions, either physiological (stress, pregnancy) or pathological (inflammation), may play a role in the fluctuation of VWF levels. Thus, the diagnosis of VWD, and in particular of type 1, may require several laboratory assessments.

The diagnosis of VWD involves the study of the family of the subject referred for investigation in order to document a convincing personal and/or familial bleeding history co-segregating with low VWF levels. Although a definite diagnosis should reasonably require that reduced levels of VWF are documented in at least two first degree family members including the proband (or even more in the case of third or lower degree of consanguinity) with bleeding, this ideal situation is rarely encountered in clinical practice. So far, minimum criteria for the diagnosis of VWD have not been agreed on. In any case, the physician must take into account many aspects, besides the strict laboratory criteria discussed below.

In order to confirm or exclude a diagnosis of VWD in a patient referred for a personal and/or family bleeding history, the first step is to exclude other common causes of bleeding. This involves the application of a simple screening procedure including

bleeding time (BT), platelet count and examination of a peripheral blood smear, APTT, and prothrombin time. If the results do not suggest a specific disorder, it is appropriate to proceed to measure VWF, given the high prevalence of this disorder. Apart from VWD, other common defects encountered include some qualitative platelet defects, such as storage pool deficiency,⁴³ or, more rarely, release reaction defects. These defects should be investigated in the presence of prolonged BT and no VWF deficiency. In the case of prolonged APTT and/or PT, assay of specific factors is indicated. BT (Ivy method) is still maintained as an important screening test, despite its recognized limitations in predicting surgical bleeding.⁴⁴ A VWF assay should be carried out even in the presence of a normal BT in a patient with a convincing bleeding history, but its prolongation is required to proceed with platelet function studies. Patients with prolonged BT and no VWF or platelet defects are encountered more often than reported. From the practical point of view, these patients usually respond to desmopressin and a test infusion is indicated.⁴⁵

Three main measurements reflect the level and/or the *activity* of VWF in plasma, namely: VWF antigen (VWF:Ag), VWF ristocetin cofactor activity

Table 7. Other tests proposed for von Willebrand's disease diagnosis.

Test	Pathophysiological significance	Diagnostic significance
Binding of VWF to collagen	VWF-collagen interaction	Correlates with VWF:RCo in type 1 VWD; some collagen preparations more sensitive to high molecular weight multimers
Closure time PFA-100	Simulates primary hemostasis after injury to a small vessel	More sensitive than BT 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	MoAb against an epitope of VWF involved in the interaction with GpIb	Correlation with VWF:RCo not confirmed; not to be used in place of VWF:RCo
ELISA-based "VWF:RCo"	Measures interaction between VWF and captured rGp Iba fragment in the presence of ristocetin	Promising new test to use instead of VWF:RCo; more data needed

Table 8. Diagnostic flow-chart for von Willebrand's disease.

1.	Consider VWD diagnosis within the context of an appropriate personal and/or family bleeding history
2.	Exclude other common hemostatic defects by performing BT, platelet count, APTT, PT. A prolonged APTT demands FVIII:C assay as a priority test
3.	If no abnormalities, irrespective of APTT and/or FVIII:C level are found proceed with VWF:RCo assay. If this assay is not available in your laboratory, perform VWF:Ag assay or VWF:CB assay. VWF:Ag < 3 U/dL suggests type 3 VWD
4.	If any of these tests is below the normal range for the ABO matched reference range, consider the diagnosis of VWD. The clinical usefulness of separate reference ranges for ABO has not been demonstrated
5.	In mild deficiencies, repeat the assay on a second occasion to confirm the diagnosis or to increase the sensitivity of the procedure in the case of a normal test in a patient with an high diagnostic suspicion
6.	Examine other family members of the proband with a possible bleeding history. Finding another member with bleeding and reduced VWF strongly confirms the diagnosis
7.	Perform VWF:Ag and VWF:RCo and FVIII:C on the same sample to look for reduced ratio VWF:RCo/VWF:Ag (a ratio < 0.7 suggest type 2 VWD) or FVIII:C/VWF:Ag (a ratio < 0.7 suggest type 2N VWD, to be confirmed by binding study of FVIII:C to patient's VWF)
8.	
9.	Perform multimeric assay using a low resolution gel. Lack of high molecular weight multimers suggests type 2A and/or 2B. Presence of a full complement of multimers suggests type 1 (or 2N, 2M). Absence of multimers in type 3

(VWF:RCo) and FVIII coagulant activity (FVIII:C) (Table 6).

The pros and cons of the different methods used for these measurements have been extensively discussed.⁴⁶ VWF:Ag is usually measured by an ELISA using polyclonal antibodies, which is suitable method for automation and robotic instrumentation. VWF:RCo is best measured by using formalin-fixed normal platelets and ristocetin at a fixed concentration (1 mg/mL, final concentration). It is a sensitive test, but its main limitation is poor inter-laboratory reproducibility. Table 7 shows other proposed tests for the diagnosis of VWD.

In the last decade many investigators have proposed VWF:CB as a substitute for VWF:RCo, since in their hands this assay was sensitive to high molecular weight multimers such as VWF:RCo, but more reproducible and easier to perform.⁴⁶ However, more studies are needed before recommending its use in clinical practice.

Table 8 summarizes a practical approach to the diagnosis of VWD based on the best scientific evidence and current clinical practice.

Conclusions

VWD is the most frequent inherited bleeding disorder. Only phenotypic diagnosis is so far easily available because of the difficulties of molecular diagnosis. Ideally, the diagnostic criteria should include all of the following: 1) bleeding symptoms, 2) low VWF level and 3) some evidence of inheritance based on either bleeding symptoms or low VWF. Any disease characterized by hemorrhage and low VWF should be considered as VWD, independently of the demonstration of a specific mutation

within the VWF gene.

The prevalence of clinically relevant VWD has not been estimated directly in the population. A figure of around 100 cases/1,000,000 population, similar to that of hemophilia A, is widely accepted, but it stems only from the limited data reported. No established criteria are available for screening the population before surgery or other risky situations and caution should be used before defining a subject with mild reduction of VWF and/or doubtful bleeding history as having VWD, without evidence of a clear personal and/or family bleeding history and consistently reduced VWF values. Repeated investigation is warranted should bleeding symptoms become more serious. Desmopressin is almost always successful in these cases.

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Treatment of inherited von Willebrand's disease

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The main goals of treatment in von Willebrand's disease (VWD) are to correct the dual defects of hemostasis caused by a reduced or abnormal von Willebrand factor (VWF), i.e. the prolonged bleeding time (BT) and the deficiency of factor VIII coagulant activity (FVIII:C). The correction of VWF defect can be achieved by two therapeutic approaches: desmopressin which can increase the endogenous VWF and/or the administration of exogenous VWF through factor VIII/VWF concentrates. Desmopressin (DDAVP), has been widely used in the management of VWD. DDAVP is safe, inexpensive and effective in about 2/3 of all VWD patients. But there are limits: it is not effective in severe forms of VWD such as type 3 and in several cases of type 1 and 2 VWD. In type 2B VWD DDAVP can induce transient thrombocytopenia. Most patients treated repeatedly with DDAVP become less responsive to therapy because tachyphylaxis may occur. VWD patients who are or become unresponsive to DDAVP require substitutive therapy with FVIII/VWF concentrates. Treated with virucidal methods, these concentrates are effective and currently safe, but the BT defect is not always corrected by them. Platelet concentrates or desmopressin can be used as adjunctive treatments when poor correction of the BT after concentrates is associated with continued bleeding. Several studies are in progress: first of all, to characterize patients with type 1 VWD better and to determine their response to DDAVP; secondly, to evaluate the pharmacokinetics of FVIII following administration of FVIII/VWF concentrates and to establish the indication for pure VWF products.

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von Willebrand's disease



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The goal of therapy in patients with inherited von Willebrand's disease (VWD) is to correct the dual defects of hemostasis, i.e. the prolonged bleeding time (BT) due to abnormal platelet adhesion and the abnormal intrinsic coagulation pathway due to low factor VIII (FVIII) levels. There are two treatments of choice in VWD, i.e. desmopressin and transfusional therapy with plasma-derived FVIII von Willebrand factor (VWF) concentrates.¹⁻³ Other forms of treatment can be considered as adjunctive or alternative to these. In 1998 an *Italian National Registry of VWD (Re.Na.Wi.)*, granted by the Italian Ministry of Health, was organized on behalf of the Italian Association of Hemophilia Centers (AICE). The aims of the Re.Na.Wi were to evaluate the retrospective natural history of VWD in Italy (> 1,000 cases) with information about the need for desmopressin and FVIII/VWF concentrates. The data collection of Re.Na.Wi is still ongoing but preliminary results have been already reported⁴ and used to update the Italian Guidelines for diagnosis and management of VWD which were published on behalf of the AICE in June 2000.⁵ In this review article the criteria of VWD treatment as proposed by the Italian Guidelines will be discussed.

Desmopressin in VWD

Desmopressin (1-deamino-8-D-arginine vasopressin, DDAVP) is a synthetic analog of vasopressin originally designed for the treatment of diabetes insipidus. DDAVP increases FVIII and VWF plasma concentrations without important side effects when administered to healthy volunteers or patients with mild hemophilia and VWD.⁶ The mode of action of DDAVP is not completely understood. Addition of DDAVP to cultured endothelial cells has no effect on VWF synthesis or secretion, so that the agent is presumed to act through an as yet unidentified second messenger.⁷ The first clin-

ical trial of DDAVP was successfully performed in 1977, with the aim of avoiding the use of blood products in mild hemophilia and VWD patients who needed dental extractions and other surgical procedures.⁸ Following these early observations, DDAVP has become widely used for the treatment of these diseases.⁹ The obvious advantages of DDAVP are that it is relatively inexpensive and carries no risk of transmitting blood-borne viruses.

DDAVP is usually administered intravenously at a dose of 0.3 µg/kg diluted in 50 mL saline infused over 30 minutes. This treatment increases plasma FVIII and VWF 3 to 5 times above the basal levels within 30 minutes. In general, high FVIII/VWF concentrations last for 6 to 8 hours. Since the responses in a given patient are consistent on different occasions, a test dose of DDAVP administered 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, several patients treated repeatedly with DDAVP become less responsive to therapy.¹⁰ The drug is also available in concentrated forms for subcutaneous and intranasal administration, which can be convenient for home treatment.⁵ Side effects of DDAVP are usually mild and include tachycardia, headache, and flushing. These symptoms 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 antidiuretic effects of DDAVP are relative rare. A few cases have been described, mostly in young children who received closely repeated infusions.¹¹ Even 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.^{12,13}

DDAVP is most effective in patients with type 1 VWD, especially those who have normal VWF in storage sites (type 1, *platelet normal*): in these patients FVIII, VWF and the BT are usually corrected within 30 min and remain normal for 6-8 hours. In other VWD subtypes, responsiveness to DDAVP is variable: a poor and short-lasting response is observed in type 1, *platelet low*.¹⁴ In type 2A, FVIII levels are usually increased by DDAVP but the BT is shortened in only a minority of cases. Desmopressin is contraindicated in type 2B, because of the transient appearance of thrombocytopenia.¹⁵ However, there have been reports on the clinical usefulness

of DDAVP in some 2B cases.^{16,17} In type 2N relatively high levels of FVIII are observed following DDAVP, but released FVIII circulates for a shorter period in patient's plasma because the stabilizing effect of VWF is impaired.¹⁸ Patients with type 3 VWD are usually unresponsive to DDAVP. However, a subgroup of these patients has been recently identified in whom FVIII becomes normal after DDAVP, even though the BT remains markedly prolonged.¹⁹ This peculiar behavior will be understood only when the characterization of the genetic defects of these patients becomes available.

The preliminary results of two large studies on the use of DDAVP in VWD are now available: a retrospective analysis on mild and severe type 1 and 2 VWD patients (n = 774) of the Italian National Registry of VWD;²⁰ and a prospective European study on clinically severe forms of type 1 and 2 VWD.²¹

Retrospective studies

Among many other parameters collected by 16 Italian Hemophilia Centers in a total number of 1,234 patients enrolled in the registry, specific information on VWD patients who could be responsive to DDAVP was available. To evaluate the use of DDAVP retrospectively in all cases of mild and severe type 1 and 2 VWD, we used the following criteria: a) *inclusion criteria*. All hereditary type 1, 2A, 2M and 2N VWD patients with a bleeding history (more than one lifelong episode of severe blood loss); b) *protocol of DDAVP test*. Test: a 0.3 µg/kg i.v. infusion was performed with BT and FVIII/VWF tested before and 0.5, 1, 2, 4 hours after DDAVP; c) *definition of response to DDAVP*: cases who, after 2 hours, showed at least 3-fold increases of baseline levels of FVIII:C and VWF:RC₀, with levels of at least 30 IU/dL and BT of 12 min or less. Among 774 VWD cases with a bleeding history enrolled in the study, 566/774 (73%) performed a DDAVP test; 408/566 (72%) were responsive to DDAVP; 206/408 (51%) used DDAVP without and 72/408 (18%) with FVIII/VWF concentrates to treat their bleeding episodes.²⁰

Prospective studies

The aims of the prospective European Multi-center study were to evaluate the biological response to DDAVP in *severe* type 1 and 2 VWD patients and to define the proportion of DDAVP-unresponsive cases who may require plasma-derived FVIII/VWF concentrates. The protocol and definition of response were similar to those of the Italian Study but the *inclusion criteria* were different: hereditary type 1 and 2 VWD with a severe bleeding history

(more than one lifelong episode of severe blood loss) and at least one of the following parameters: BT > 15 min; VWF/RCo < 10 IU/dL; FVIII:C < 20 IU/dL. Patients with type 3 and type 2B VWD, with acquired von Willebrand's syndrome, those aged < 12 and > 65 years, patients with cardiovascular diseases and epilepsy or with previous reactions with DDAVP were excluded. Among a total of 957 VWD patients followed by five Hemophilia Centers, 116/957 (12%) satisfied the inclusion criteria and 67/957 (7%) were enrolled in this study after giving their informed consent. Only 18/67 (27%) clinically severe type 1 and 2 VWD patients were responsive to DDAVP, suggesting that a DDAVP infusion test should always be performed in patients with clinically severe VWD to exclude the need for replacement therapy with concentrates.²¹

The apparent conflicting results obtained by these studies are certainly due to the different inclusion criteria. However, these data might suggest that, though DDAVP has been used in VWD since 1997, its effects and limits are not completely clarified especially when its administration must be repeated for several days during surgery.

Other non-transfusional therapies for VWD

Two other types of non-transfusional therapies are used in the management of VWD, i.e. antifibrinolytic amino acids and estrogens.

Antifibrinolytic amino acids

Antifibrinolytic amino acids are synthetic drugs which interfere with the lysis of newly formed clots by saturating the binding sites on plasminogen, thereby preventing this latter's attachment to fibrin and making plasminogen unavailable within the forming clot. Epsilon aminocaproic acid (50 mg/kg four times a day) and tranexamic acid (25 mg/kg three times a day) are the most widely used antifibrinolytic amino acids. Both medications can be administered orally, intravenously or topically and are useful as adjuncts in the management of oral cavity bleeding, epistaxis, gastrointestinal bleeding and menorrhagia. As drugs that inhibit the fibrinolytic system, they carry a potential risk of thrombosis in patients with an underlying prethrombotic state. They are also contraindicated in the management of urinary tract bleeding.

Estrogens

Estrogens increase plasma VWF levels, but the response is so variable and unpredictable that 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.

FVIII/VWF concentrates in VWD

VWD patients who are or become unresponsive to DDAVP must be treated with FVIII/VWF concentrates. For many years cryoprecipitate has been the mainstay of replacement therapy but, after the introduction of virucidal methods, FVIII/VWF concentrates have been considered much safer than cryoprecipitate. In fact, these concentrates consistently correct the FVIII:C defect and contain a large amount of VWF, except for monoclonally purified concentrate.¹⁻³ Several different FVIII/VWF concentrates have been produced from plasma by pharmaceutical companies and are now commercially available: the characteristics of the four FVIII/VWF concentrates registered in Italy, as reported in the Italian guidelines for VWD diagnosis and therapy,⁴ are summarized in Table 1. These plasma-derived FVIII/VWF concentrates have been tested in both pharmacokinetic and efficacy studies by many authors.²²⁻³² A recombinant VWF concentrate has also been produced: however, it has been tested in animals but not in patients with VWD.³³

Pharmacokinetic studies

The most extensive studies about *in vitro* and pharmacokinetic analysis of FVIII/VWF concentrates were published in 1992.²⁴ Mannucci's study was the first and unique comparison, in a complete cross-over, randomized trial, of the four virus-inactivated FVIII/VWF concentrates most used to treat severe VWD. This approach was further approved by the *Scientific Standardization Committee on VWF of the International Society of Haemostasis* and used to prepare the recommendations for testing new FVIII/VWF concentrates.²⁵ In that study, none of the four FVIII/VWF concentrates tested showed a VWF protein with an intact multimeric structure similar to that of normal plasma or of cryoprecipitate, as detected by the correct analytical system to search for VWF high molecular weight multimers (i.e. low resolution agarose gels). Despite this abnormal multimeric structure of the VWF contained in the tested commercial products, all four FVIII/VWF concentrates were equally effective in increasing FVIII/VWF activities in type 3 VWD cases as proven by pharmacokinetic studies. All were effective in obtaining normal and sustained levels of FVIII:C post-infusion, although peak levels were more delayed for one of the concentrates which is devoid of FVIII:C.²⁴ VWF antigen (VWF:Ag) and VWF

Table 1. FVIII/VWF concentrates commercially available in Italy (as from the *Guidelines for the diagnosis and treatment of von Willebrand's disease approved by the Italian Association of Hemophilia Centers*).

Products (manufacturers)	Purification	Viral inactivation	Specific activity* (U/mg prot.)	VWF:RCo/ Ag° (ratio)	VWF:RCo/ FVIII° (ratio)	Other proteins
Emoclot D.I. (Kedrion)	Ion exchange chromatography	Solv./Det., +30 min. a 100°C	> 80	0.61	1.16	Albumin -
Fanndi (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. + Vapor 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 manufacturers on different concentrate stocks could be reported.

ristocetin cofactor (VWF:RCo) activities were also normalized by all FVIII/VWF concentrates. However no FVIII/VWF concentrate normalized the BT in a sustained fashion, indicating once more that BT is not coincident with VWF:RCo.²⁴ In fact all FVIII/VWF concentrates are always able to correct the VWF:RCo, while BT is not always corrected.

Pharmacokinetic studies of single FVIII/VWF concentrates have also been organized: Menaché *et al.* studied the pharmacokinetics of VWF and FVIII in severe type 3 VWD after the infusion of a VWF concentrate devoid of FVIII and they were able to estimate the rate of FVIII:C synthesis in these patients.³⁰ An additional cross-over pharmacokinetic study has been organized on behalf of the European Community and data will be available within the end of 2001.³¹ The aims of this European multicenter study is to evaluate a new plasma-derived concentrate devoid of FVIII (VWF-SD-35-DH) versus Haemate-P or Innobrand (France) in patients with clinically severe VWD unresponsive to DDAVP. Data from another large multicenter pharmacokinetic cross-over study performed with Alphanate-SD versus Alphanate-SD/HT in different VWD American and Italian patients will be published soon.³² Once again, these two FVIII/VWF concentrates can always correct FVIII/VWF activities but do not always normalize BT (BT normalized = 33%; partially corrected = 43%; unchanged = 24%).

Efficacy studies and clinical practice

It is well known in clinical practice that clinical hemostasis following FVIII/VWF concentrates can be achieved in all types of VWD regardless of whether the BT is corrected. This is particularly true in the management of VWD patients during surgery, as shown by the experience of several hematologists and by the results of the recent effi-

cacy multi-center study. Haemate-P was very effective in the management of both pediatric and adult patients with VWD.^{28,29} Alphanate was effective in treating and preventing bleeding episodes: according to the results of the Alphanate Study Group, 73% of bleeding episodes were solved by a single infusion of concentrate and hemostatic control during surgery was provided even in the absence of BT correction. However, physicians who are giving FVIII/VWF concentrate for several days to prevent bleeding after surgery should be aware of the delayed response of FVIII:C, as originally observed following cryoprecipitate. Another multi-center efficacy study by using the VWF concentrate devoid of FVIII is now in progress it will be interesting to evaluate whether or not this concentrate can correct BT without inducing excessive levels of FVIII:C.³¹

Specific therapeutic approaches

Mucosal and gastrointestinal bleeding

Mucosal bleeding occurs very frequently in VWD and may last for a long time. In the case of gastrointestinal bleeding, VWD patients can stay in hospital for many days and sometimes require months of treatment. We have had several patients with this situation and most of them were managed with daily or every other day infusions of FVIII/VWF concentrates. When bleeding persists despite replacement therapy, other therapeutic options are available. DDAVP, given after FVIII/VWF concentrates, may shorten or normalize the BT in patients with type 3 VWD as shown in the study with DDAVP after cryoprecipitate.³⁴ Platelet concentrates (given before or after FVIII/VWF concentrates, at doses of 4-5×10¹¹ platelets) may achieve similar effects in patients unresponsive to concentrates alone, both in terms of BT correction and

bleeding control, as shown in the study of platelet concentrates after cryoprecipitate.³⁵ These data emphasize the important role of platelet VWF in establishing and maintaining primary hemostasis.

VWD during pregnancy and delivery

During pregnancy VWF and FVIII levels tend to rise in type 1 and 2 VWD but this rise does not occur until the 10-11th weeks of gestation. No significant changes occur in patients with type 3 VWD. Since improvements in VWF and FVIII levels during pregnancy are variable, patients should be monitored during pregnancy and for several weeks after delivery when levels fall rapidly and may cause late bleeding.³⁶ In type 1 VWD, FVIII levels are the best predictor of the risk of bleeding at delivery. The risk of bleeding is minimal when FVIII is > 50 U/dL but can be significant when it is lower than 20 U/dL.³⁶ Careful surgical hemostasis along with effective uterine contraction usually compensates for a prolonged BT. In type 3 VWD, characterized by prolonged BT and low FVIII levels, replacement therapy with concentrates is necessary. Thrombocytopenia may develop or be aggravated in patients with type 2 B VWD during pregnancy,^{37,38} but it is not clear whether thrombocytopenia exacerbates clinical bleeding.

Patients with type 3 VWD and allo-antibodies

For the rare patients with type 3 VWD who develop anti-VWF alloantibodies after multiple transfusions, the infusion of VWF concentrates not only is ineffective, but may also cause post-infusion anaphylaxis due to the formation of immune complexes.⁴⁰⁻⁴⁴ These reactions may be life-threatening.⁴³ To overcome this reaction, a patient undergoing emergency abdominal surgery was treated with recombinant FVIII, because this product, being completely devoid of VWF, did not cause anaphylactic reactions. Due to the very short half-life of FVIII devoid of its VWF carrier, recombinant FVIII had to be administered by continuous i.v. infusion, at very large doses in order to be sufficient to maintain FVIII levels above 50 U/dL for 10 days after surgery.⁴⁴

Recommendations and future expectations

Thanks to improved diagnosis, patients with different types of VWD can nowadays be treated with desmopressin and/or FVIII/VWF concentrates with more appropriateness. The treatments of choice and the alternative and adjunctive therapies in each VWD subtype as recommended by the Italian

Table 2. Management of different types and subtypes of von Willebrand's disease.

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

Table 3. Doses of factor VIII/VWF concentrates recommended in von Willebrand's disease patients unresponsive to desmopressin.

<i>Type of bleeding</i>	<i>Dose (IU/kg)</i>	<i>No. of infusions</i>	<i>Objectives</i>
Major surgery	50	Once a day or every other day	Maintain factor VIII > 50 IU/dl until healing is complete
Minor surgery	30	Once a day or every other day	Factor VIII >30 IU/dl until healing is complete
Dental extractions	20	Single	Factor VIII >30 IU/dl for up to 6 hours
Spontaneous or post-traumatic bleeding	20	Single	

Association of Hemophilia Centers are summarized in Table 2. The dosage of FVIII/VWF concentrates to be used in VWD patients unresponsive to DDAVP are also shown (Table 3). The Italian guidelines for VWD management further suggest that patients who are responsive to desmopressin should be identified immediately at the time of diagnosis. Those who are unresponsive to DDAVP and who may require plasma-derived FVIII/VWF concentrates should be vaccinated against the major viral infections.⁵

Two issues about VWD management are still open at the beginning of the third millennium: i) the identification and characterization of the patients who are responsive to DDAVP; and ii) the use of more purified VWF concentrates with or without FVIII. The first issue may well be resolved

by a three-year International Project entitled *Molecular and Clinical Markers for Diagnosis and Management of type 1 VWD*, sponsored by the European Community. In this project, started in September 2000, twelve European Hemophilia centers will enrol 200 families with type 1 VWD and will correlate the phenotypic data with molecular abnormalities, inheritance pattern and response to DDAVP. The second issue will be explored by the ongoing pharmacokinetic and efficacy studies with FVIII/VWF concentrates with or without FVIII:C. The pharmacokinetics of FVIII:C following FVIII/VWF concentrates in VWD is important to establish whether or not there are indications for pure VWF products devoid of FVIII:C, will come from the cooperative study entitled *Optimizing Orphan Drug Therapy in Severe Forms of VWD*. The data from this study, sponsored by the European Community started in 1997, will be available within 2001.

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Treatment of acquired von Willebrand's syndrome

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Acquired von Willebrand's syndrome (AVWS) is a rare acquired bleeding disorder similar to the congenital von Willebrand's disease (VWD) in terms of laboratory findings. Diagnosis of AVWS can be very difficult and treatment has usually been empirical. Compared to the congenital VWD (0.1-1% of the general population), the cases of AVWS reported in the literature (about 300) since 1968 are likely to be underestimate the true prevalence. However, large retrospective or prospective studies are not available. Recently, the data from an international registry on 186 AVWS cases, provided retrospectively by Departments of Hematology and Oncology and Hemophilia Centers world-wide, have been used to prepare guidelines for the diagnosis and management of this acquired disorder. AVWS cases are associated with lymphoproliferative or myeloproliferative disorders, cardiovascular diseases, neoplasia and other miscellaneous clinical conditions. Bleeding episodes can usually be managed by desmopressin, FVIII/VWF concentrates, immunoglobulins, plasmapheresis, steroids and immunosuppressive drugs but none of these therapeutic approaches is always effective in all AVWS cases. Therefore, treatment must be chosen in each patient according to the underlying disorder as well as to the type and severity of bleeding.

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Key words: acquired von Willebrand's syndrome, anti-factor VIII/von Willebrand factor antibodies, therapy, desmopressin, factor VIII/von Willebrand factor concentrates, immunoglobulins, steroids, immunosuppressive agents.

von Willebrand's disease



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Acquired von Willebrand's syndrome (AVWS) is a rare bleeding disorder similar to the congenital disease in terms of laboratory findings, being characterized by a prolonged bleeding time (BT) and low plasma levels of factor VIII von Willebrand factor (FVIII/VWF). AVWS usually occurs in subjects without a personal or family history of bleeding.¹ Since the original description of a case in a patient with systemic lupus erythematosus reported in 1968,² about 300 cases of AVWS have been described in the literature. The complete list of references about AVWS can be found on the Internet (<http://www.med.unc.edu/isth/welcome.htm>). Several review articles about AVWS have been also published³⁻⁶ but no retrospective or prospective analyses are available. Only recently, the data from an international registry on 186 AVWS cases, provided retrospectively by Departments of Hematology and Oncology and Hemophilia Centers world-wide, have been used to prepare guidelines for the diagnosis and management of this acquired disorder.⁷ In this review article these published recommendations on the management of AVWS will be discussed.

Underlying disorders associated with AVWS

AVWS cases are mainly associated with lymphoproliferative disorders, myeloproliferative disorders, solid tumors, immune diseases, cardiovascular diseases and other miscellaneous conditions including viral infections and some drugs.³⁻⁷ The list of underlying disorders associated with AVWS is summarized in Table 1. The recent results of an international registry on 186 additional cases of AVWS from only 50 Centers seems to suggest that AVWS is more frequent than expected in lymphoproliferative (48%) and myeloproliferative (15%) disorders being present also in cardiovascular diseases (21%), solid tumors (5%), immunologic dis-

Table 1. List of underlying disorders associated with AVWS.

<i>Lymphoproliferative disorders</i>
Chronic lymphocytic leukemia
Non-Hodgkin's lymphoma
Hairy-cell leukemia
Monoclonal gammopathy of undetermined significance
Multiple myeloma
Waldenström's macroglobulinemia
<i>Myeloproliferative disorders</i>
Essential thrombocythemia
Polycythemia vera
Chronic granulocytic leukemia
Acute myelocytic leukemia
Acute myelomonocytic leukemia
<i>Solid tumors</i>
Wilms' tumor (nephroblastoma)
Adenocarcinoma of the stomach
Adrenal cortical carcinoma
Peripheral neuroectodermal tumor
<i>Immunological disorders</i>
Systemic lupus erythematosus
Hypothyroidism
Scleroderma
Mixed connective tissue disease
Graft-versus-host disease
<i>Cardiovascular diseases</i>
Aortic stenosis
Mitral valve prolapse
Congenital cardiac defects
Gastrointestinal angiodysplasia
<i>Miscellaneous</i>
DRUGS
Antibiotics:
Ciprofloxacin,
Griseofulvin
Anticonvulsivants:
Valproic acid
Plasma Volume Expander:
Hydroxyethyl starch
VIRAL DISEASES
Epstein-Barr virus
OTHER DISEASES
Uremia
Hydatid cyst
Ehlers-Danlos syndrome
Hemoglobin E β^0 thalassemia

Table 2. Pathogenetic mechanisms of AVWS.

- specific auto-antibodies;
- non-specific antibodies that form circulating immune-complexes and favor VWF clearance by Fc-bearing cells;
- absorption onto malignant cell clones;
- increased proteolytic degradation;
- loss of high VWF multimers by high shear stress conditions.

Note that a reduced VWF synthesis has been demonstrated in AVWS associated with hypothyroidism.

orders (2%) and other miscellaneous conditions (9%). The distribution of these underlying disorders as obtained from both the literature and the registry is summarized in Figure 1.

Diagnosis and pathogenesis of AVWS

The diagnosis of AVWS is usually based on laboratory findings typical of VWD in patients without a personal or family history of bleeding. Many attempts have been made to develop laboratory tests to demonstrate the presence of an inhibitor against VWF. Unlike other acquired hemostatic defects such as factor VIII deficiency (i.e., acquired hemophilia A), inhibitory antibodies against FVIII/VWF can be demonstrated in only a relatively small proportion (10-20%) of cases with AVWS and therefore the pathogenetic mechanism of the VWF deficiency often remains undetermined. It has been proven that VWF is normally synthesized in most cases of AVWS, except for those with hypothyroidism which are characterized by decreased synthesis of VWF.³⁻⁷ The normally synthesized VWF is removed at an accelerated rate from plasma through five possible pathogenetic mechanisms (Table 2). However none of the proposed mechanisms appears to be disease-specific: the same mechanism can be responsible for AVWS in many different diseases and more than one mechanism can induce AVWS in one of the underlying disorders known to be associated with AVWS.³⁻⁷

Management of AVWS: general criteria

In the absence of a consistent pathogenetic mechanism, the management of AVWS has usually been empirical. The goals of treatments are threefold: a) to treat the underlying disease whenever possible; b) to control any bleeding episodes; c) to prevent bleeding whenever an invasive procedure is deemed necessary. In several disorders associated with AVWS, surgery, chemotherapy, radiotherapy or immunosuppressive drugs can sometimes control the underlying disease, with resolution of the bleeding diathesis and normalization of the laboratory abnormalities.³⁻⁷ In others, such as AVWS associated with a monoclonal gammopathy of undetermined significance (MGUS), the underlying disease is usually not treated. Hence the best approach to stop bleeding episodes and/or to prevent bleeding during surgery must be found.⁸

Treatment of the underlying disorders

An extensive search for one of the underlying disorders usually associated with AVWS (Table 1) is recommended when a new case of AVWS is diagnosed. Treatment of the underlying disorder usually results in the correction of the hemostatic defects causing the AVWS.³⁻⁷ In fact, spontaneous regres-

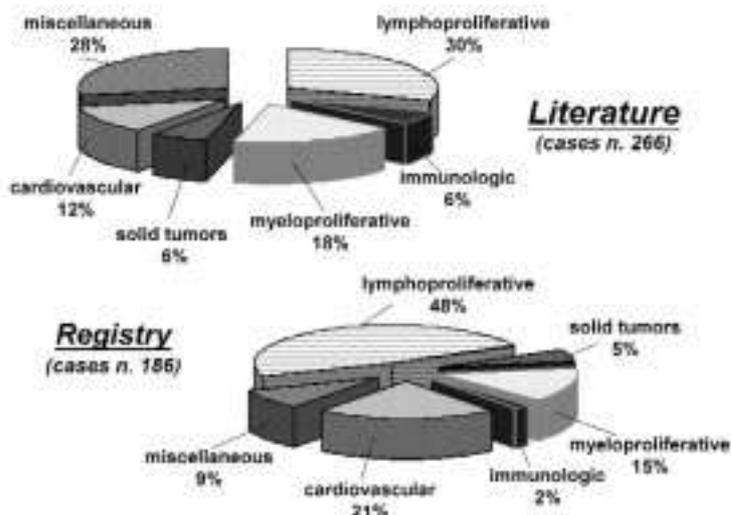


Figure 1. Underlying disorders associated with AVWS: comparison between data from patients reported in the literature (n=266) and those from cases analyzed by the International Registry (n=186). The distribution (%) of AVWS in the following six categories of underlying disorders is described: lymphoproliferative, myeloproliferative, solid tumors, immunologic, cardiovascular, miscellaneous.

sion of the hemostatic defects has been observed only occasionally.⁹ Successful approaches have included surgical removal of a solid tumor¹⁰⁻¹² or surgical repair of a heart valve defect.¹³ Chemotherapy used to induce remission of hemato-oncologic disorders may correct AVWS¹⁴⁻¹⁶ and the AVWS associated with high platelet counts also usually responds very well to cytoreduction.¹⁷ Radiotherapy can also be effective in some cases.¹⁸ Hormone replacement corrects the AVWS associated with hypothyroidism.¹⁹ In the case of drug-induced AVWS, discontinuation of the drug responsible is usually sufficient to reverse the hemostatic defect

of AVWS.^{20,21} Unfortunately, in a few cases of AVWS the underlying disorder is not identified²² or is resistant to therapy. In these cases the best approach to stop bleeding episodes and/or to prevent bleeding during surgery must be found.⁸

Therapeutic approaches used to stop bleeding in AVWS

Many different therapeutic approaches have been used in AVWS, including mainly desmopressin, factor VIII/VWF concentrates, high-dose immunoglobulins but also plasmapheresis, steroids and immunosuppressive drugs.³⁻⁷ No treatment can be effective in all AVWS cases: often, more than one

Table 3. Different therapeutic approaches in 186 cases with AVWS from the Registry.* Number (%) of patients who respond out of treated.

Associated disorders: Case number (%):	Lymphoproliferative 89 (48)	Myeloproliferative 29 (15)	Neoplasia 9 (5)	Immunologic 4 (2)	Cardiovascular 39 (21)	Miscellaneous 16 (9)	Total 186 (100)
DDAVP	26/59 (44)	3/14 (21)	3/4 (75)	1/3 (33)	3/30 (10)	2/9 (22)	38/119 (32)
FVIII/VWF concentrate	28/56 (50)	2/14 (14)	6/6 (100)	—	4/30 (13)	2/9 (22)	42/115 (37)
I.V. immunoglobulin	18/48 (37)	—	2/2 (100)	1/2 (50)	0/10 (0)	0/1 (0)	21/63 (33)
Plasmapheresis	6/30 (20)	—	— (0)	— (19)	0/2	—	6/32
Corticosteroids	10/31 (32)	—	0/1 (0)	—	2/25 (8)	0/6 (0)	12/63 (19)
Immunosuppressive agents or chemotherapy	17/47 (36)	5/14 (36)	1/2 (50)	—	—	0/3 (0)	23/66 (35)

*More than one approach is used in the same patient (modified from ref. #7).

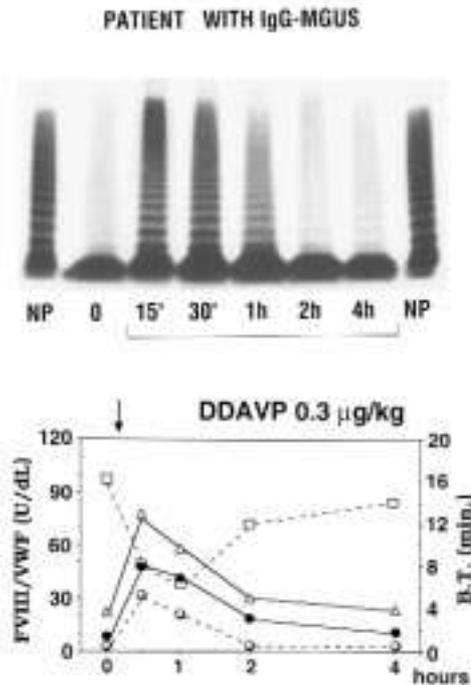


Figure 2. The effects of a standard dose of desmopressin (DDAVP) in patients with AVWS associated with IgG-MGUS. Upper panel: the multimeric pattern of VWF as shown by low-intermediate resolution SDS-1.2% LGT agarose gels is described in comparison with normal plasma (NP) before, 15 min, 30 min and 1, 2, and 4 hours following DDAVP. Note that the high molecular weight multimers are present for about 1 hour after DDAVP. Lower panel: bleeding time (BT, open squares), factor VIII (open triangles), VWF:Ag (closed circles), VWF:RCo (open circles) levels before and after DDAVP at times corresponding to those in the upper panel. Note that BT is shortened and FVIII/VWF activities are significantly increased or normalized only for a short time.

therapeutic approach must be used to stop bleeding (Table 3). Despite many reports, there is only one study which investigate three of the most used therapeutic approaches — desmopressin, FVIII/VWF concentrates and high-dose immunoglobulins — in the same group of ten patients.⁸ These patients are characterized by an AVWS associated with monoclonal gammopathy of undetermined significance (MGUS) and their bleeding problems were managed by using one or more of the therapeutic approaches.⁸ As examples, the effects on BT and on FVIII/VWF activities including multimeric pattern are summarized in these patients with AVWS associated with MGUS according to the type (IgG-MGUS versus IgM-MGUS) of monoclonal paraproteins (Figures 2-7).

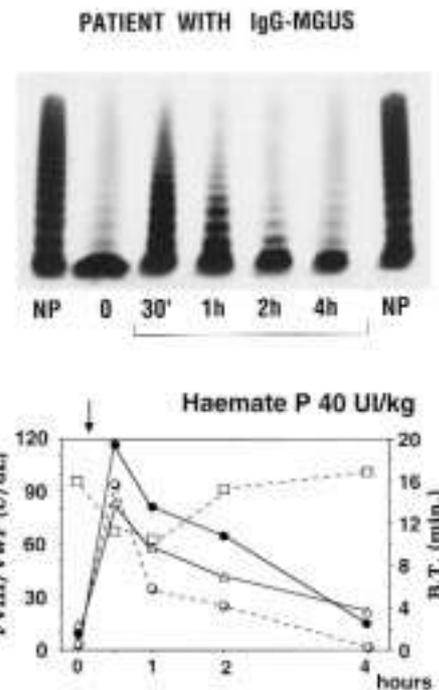


Figure 3. The effects of a standard dose (40 U/kg) of Haemate-P, a commercially available FVIII/VWF concentrate, in patients with AVWS associated with IgG-MGUS. Upper panel: the multimeric pattern of VWF as shown by low-intermediate resolution SDS-1.2% LGT agarose gels is described in comparison with normal plasma (NP) before and 0.5, 1, 2, 4 hours following Haemate-P. Note that a significant increase of the intermediate-high molecular weight multimers is present for a short time after Haemate-P. Lower panel: bleeding time (BT, open squares), factor VIII (open triangles), VWF:Ag (closed circles), VWF:RCo (open circles) levels before and after Haemate-P at times corresponding to those in the upper panel. Note that BT is shortened and FVIII/VWF activities are significantly increased or normalized only for a short time.

Desmopressin

Since, by definition, VWF is usually present in normal concentrations in cellular compartments of patients with AVWS, DDAVP can induce an appropriate release of normal and functional VWF and can restore normal levels of FVIII/VWF in plasma. DDAVP is not very expensive and does not carry any risks of blood-borne infections which could be particularly dangerous in patients with the underlying diseases associated with AVWS. The main problems of DDAVP in AVWS are related to the short half-life of FVIII/VWF, due to one of the mechanisms interfering with VWF survival in AVWS. DDAVP can shorten BT and increase plasma FVIII/VWF properties only transiently in AVWS with

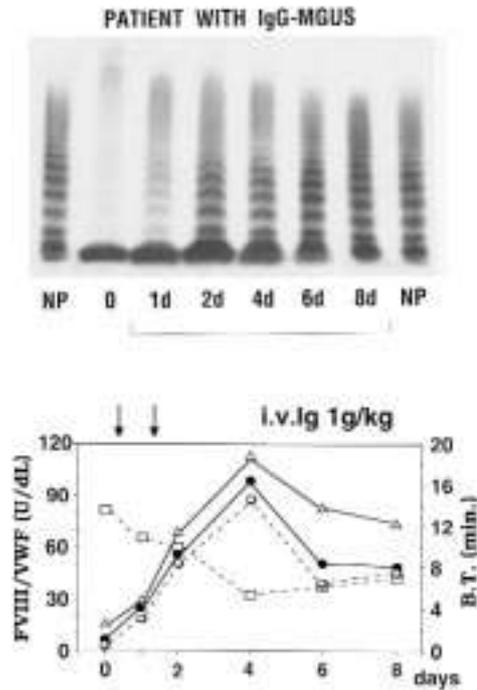


Figure 4. The effects of a standard dose (1 g/kg daily for two days) of IV-Ig in patients with AVWS associated with IgG-MGUS. Upper panel: the multimeric pattern of VWF as shown by low-intermediate resolution SDS-1.2% LGT agarose gels is described in comparison with normal plasma (NP) before and 1, 2, 4, 6 and 8 days following IV-Ig. Note that all the high molecular weight multimers progressively increase until day 4th and then the multimers remain constant until at least day 8th. Lower panel: bleeding time (BT, open squares), factor VIII (open triangles), VWF:Ag (closed circles), VWF:RCo (open circles) levels before and after IV-Ig at times corresponding to those in the upper panel. Note that BT shortened and FVIII/VWF activities normalized progressively with a delay of about two days and can remain within normal ranges for at least 8 days.

a half-life shorter than that of congenital VWD.²³ The DDAVP response pattern has been recently re-evaluated in ten additional cases affected by MGUS and results are very similar.⁸ Given the extreme heterogeneity of the mechanisms inducing AVWS, it is not surprising that the response to DDAVP can be quite variable from patient to patient even in cases with the same underlying disorder. This is why an infusion trial, with BT and FVIII/VWF activities measured before and following DDAVP administration, is indicated in every patient with a new diagnosis of AVWS. Desmopressin (DDAVP) was effective in only 32% of cases tested as shown in the registry.⁷ Apparently, DDAVP was more effective in AVWS associated with LPD (44%) than other

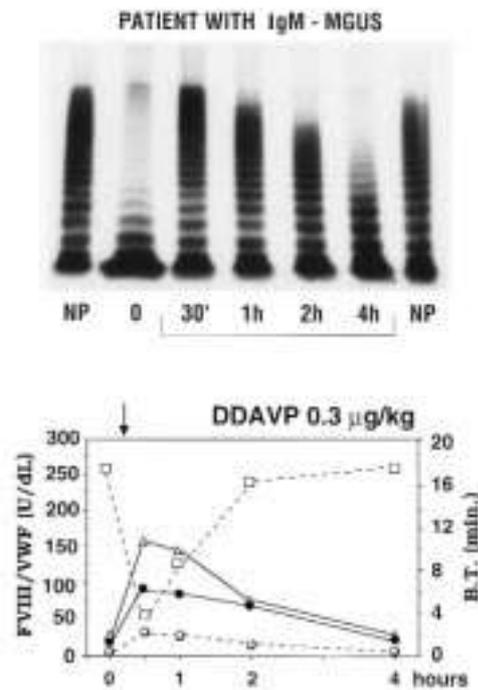


Figure 5. The effects of a standard dose of DDAVP in patients with AVWS associated with IgM-MGUS. Upper panel: the multimeric pattern of VWF as shown by low-intermediate resolution SDS-1.2% LGT agarose gels is described in comparison with normal plasma (NP) before, 0.5, 1, 2 and 4 hours following DDAVP. Note that a relative percentage of the intermediate-high molecular weight multimers are present until 1-2 hours after DDAVP. Lower panel: bleeding time (BT, open squares), factor VIII (open triangles), VWF antigen (closed circles), VWF:RCo (open circles) levels before and after DDAVP at times corresponding to those in the upper panel. Note that BT is shortened for about 1 hour and FVIII/VWF activities are significantly increased or normalized only for a short time.

underlying disorders but it is interesting to note that DDAVP was also effective in reducing bleeding in the other disorders associated with AVWS (Table 3). As an example, the BT with FVIII/VWF activities including multimeric pattern in patients with IgG-MGUS and IgM-MGUS before and after DDAVP are reported in Figures 2 and 5.

FVIII/VWF concentrates

Compared to DDAVP, plasma FVIII/VWF concentrates are more expensive and may carry some risks, though remote, of possible blood-borne infections. Also in case of FVIII/VWF concentrates, the half-life of the FVIII/VWF activities is shortened by one of the mechanisms causing AVWS. Therefore the efficacy of these concentrates must be proven every

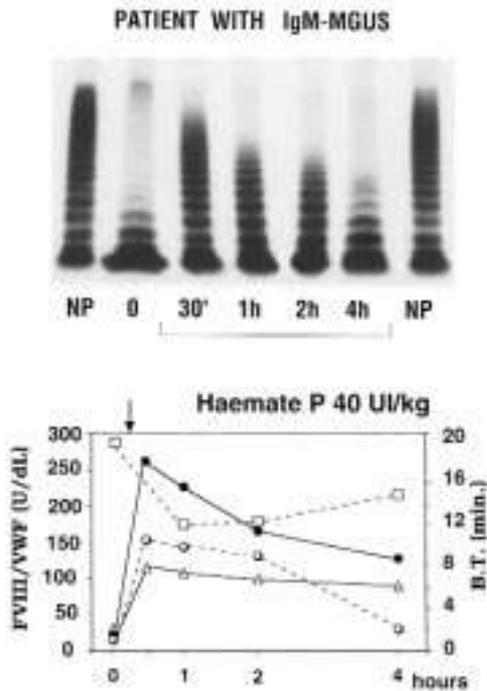


Figure 6. The effects of a standard dose (40 U/kg) of Haemate-P, a commercially available FVIII/VWF concentrate, in patients with AVWS associated with IgM-MGUS. Upper panel: the multimeric pattern of VWF as shown by low-intermediate resolution SDS-1.2% LGT agarose gels is described in comparison with normal plasma (NP) before, 0.5, 1, 2 and 4 hours following Haemate-P. Note that a significant increase of the intermediate-high molecular weight multimers is present until 1-2 hours after Haemate-P. Lower panel: bleeding time (BT, open squares), factor VIII (open triangles), VWF:Ag (closed circles), VWF:RCo (open circles) levels before and after Haemate-P at times corresponding to those in the upper panel. Note that BT shortened for 2-4 hours and FVIII/VWF activities are also significantly increased or normalized for 2-4 hours.

time by serial measurements of FVIII/VWF activities following administration of the concentrates. FVII/VWF concentrates were also used in many patients reported in the international registry with AVWS and they were effective in 37% of total AVWS cases.⁷ In the cases of solid tumors and LPD the FVIII/VWF concentrates were effective in, respectively, 100% and 50% of the patients who were treated with them (Table 3). As an example, the BT with the FVIII/VWF activities including multimers in patients with IgG-MGUS and IgM-MGUS before and after 40 U/kg of Haemate-P, one of the most used FVIII/VWF concentrates, are reported, respectively, in Figures 3 and 6.

High dose immunoglobulins

Much experience has been gathered in the treatment of AVWS with high dose immunoglobulins

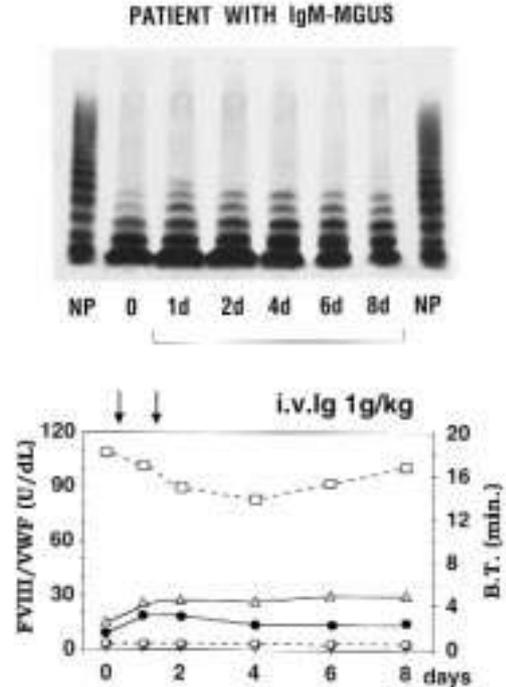


Figure 7. The effects of a standard dose (1 g/kg day for two days) of IV-Ig in patients with AVWS associated with IgM-MGUS. Upper panel: the multimeric pattern of VWF as shown by low-intermediate resolution SDS-1.2% LGT agarose gels is described in comparison with normal plasma (NP) before and 1, 2, 4, 6, 8 days following IV-Ig. Note that there are no significant changes in the VWF multimers. Lower panel: bleeding time (BT, open squares), factor VIII (open triangles), VWF antigen (closed circles), VWF:RCo (open circles) levels before and after IV-Ig at times corresponding to those in the upper panel. Note that BT is not significantly modified and FVIII/VWF activities are increased but very slightly.

(IV-Ig) since the early experiences by Maciak.²⁴ IV-Ig can be effective in AVWS associated with MGUS and in other underlying disorders characterized by inhibiting activities against VWF. It has been proven that two single daily IV-Ig infusions (short-term therapy) can induce a prompt and sustained increase of FVIII/VWF activities and shortening of the BT for at least 15 days in all IgG-MGUS patients but not in IgM-MGUS.⁸ Repeated doses of IV-Ig given every 15-21 days (long-term therapy) produced consistent responses in FVIII/VWF measurements and clinical remission of the bleeding diathesis in AVWS associated with IgG-MGUS.^{8,25} The mechanisms by which IV-Ig exert their action is not completely understood: one possibility is that they prevent the clearance of inhibitor-VWF complex from the circulation by blocking Fc-receptors.

From the data of the international registry, IV-Ig were effective in 33% of patients, especially in LPD (37%) and solid tumors (100%). As reported in the literature, the efficacy of IV-Ig seems to correlate very well with the presence of positive anti-FVI-II/VWF inhibitors:²⁶ in the registry IV-Ig response was found in 75% of patients with LPD and solid tumors who were characterized by the presence of anti-VWF antibodies. As an example, the BT with the FVIII/VWF activities including multimers in patients with IgG-MGUS and IgM-MGUS before and after high-dose IV-Ig, are reported, respectively, in Figures 4 and 7.

Additional therapeutic approaches

Thirty-two patients, of whom 30 had associated LPD, were treated with plasmapheresis or extracorporeal immunoadsorption, but this approach was effective in only 6/30 of the LPD cases (Table 3). Corticosteroids were used in 63 cases and were effective in 12% of total cases and in 32% of LPD patients (Table 3). The apparently high response to immunosuppressive agents (35%), especially in LPD (36%), MPD (36%) and solid tumors (50%), is mainly related to the use of drugs (chemotherapy with steroids and other immunosuppressive agents) given to treat the underlying conditions associated with AVWS. This is not surprising because it is

in perfect agreement with one of the goals of AVWS treatment, i.e. to treat the underlying disease whenever possible.

Recommendations

The recommendations for AVWS treatment are derived from those approved by the *Scientific Standardization Committee on VWF of the International Society of Thrombosis and Hemostasis* as already published.⁷ In the case of excessive bleeding, surgery or invasive procedures in a patient with definite AVWS the physicians must consider the proposed flow-chart for the management of AVWS shown in Table 4.

DDAVP is the first choice because AVWS patients show normal VWF in cellular compartments (endothelial cell and platelets) and, in the registry, this management was proven to be effective in 1/3 of the treated cases with AVWS (Table 3). Since the half-life of FVIII/VWF can vary from patient to patient, an infusion trial with DDAVP with BT, FVI-II:C and VWF:RCo measured before and 1, 2, 4 hours following DDAVP is recommended in every new case of AVWS to test the patient's response to this agent. During treatment, especially in the case of repeated DDAVP infusions, FVIII/VWF activities must be always measured before and after infusions to make sure that DDAVP is still effective. Only in the case of proven failure of DDAVP treatment, should other options be considered.

Plasma-derived FVIII/VWF concentrates are the second choice, because they have been proven to be effective in more than 1/3 of the AVWS patients tested in the registry (Table 3). Considering the high costs and the possible risks, albeit remote, of blood-borne infections, their use is recommended only in proven DDAVP-unresponsive cases. As for DDAVP, also in the case of concentrates, measurements of FVIII/VWF activities are required to adjust dosage and time of infusions. In the case of major surgery, an infusion trial can be justified to know the half-life of VWF in the patient and to select the appropriate dosage in advance. Dosages can vary according to the response from 50 to 100 U/kg or more. During treatment (especially in the case of repeated infusions), FVIII/VWF activities must always be measured before and after infusions.

Treatment with high-dose intravenous immunoglobulin is the third choice. Immunoglobulins must be used in specific cases such as patients with proven anti-FVIII/VWF inhibitors or those unresponsive to DDAVP and FVIII/VWF concentrates. FVIII/VWF activities must be measured before and once a day for 7-15 days following infusions to

Table 4. Flow chart for management of bleeding in AVWS. *

Problems	Excessive bleeding, surgery or invasive procedures in a patient with AVWS.
Before treatment	Make sure of a correct diagnosis of AVWS. Try to identify patients with or without anti-FVIII-VWF activities (inhibitors).
First choice	Desmopressin (DDAVP). When possible, an infusion trial is recommended During treatment, measure FVIII/VWF activities always before and after infusions.
Second choice	Factor VIII/VWF concentrates. In case of surgery, an infusion trial can be justified Dosage can vary according to the response from 50 to 100 U/Kg or more. During treatment, measure FVIII/VWF activities before and after infusions.
Third choice	High-dose i.v. IG (only in specific cases): patients with proven inhibitors or cases unresponsive to DDAVP or FVIII/VWF concentrates. Measure FVIII/VWF activities before and once a day for 7-15 days following infusions.
Additional options	Plasmapheresis and extracorporeal immunoadsorption Steroids and immunosuppressive agents (long term therapy).

*Modified from ref. #6.

demonstrate efficacy. In other underlying disorders associated with AVWS without inhibitors, the positive effects of IV-Ig remain to be proven. Given the high costs of such treatment, IV-Ig must be reserved to AVWS cases with positive anti-FVIII/VWF inhibitors and/or must be tested in bleeding patients who were proven to be unresponsive to DDAVP and FVIII/VWF concentrates.

Plasmapheresis and extracorporeal immunoadsorption or steroids and immunosuppressive agents can be considered additional therapeutic approaches.

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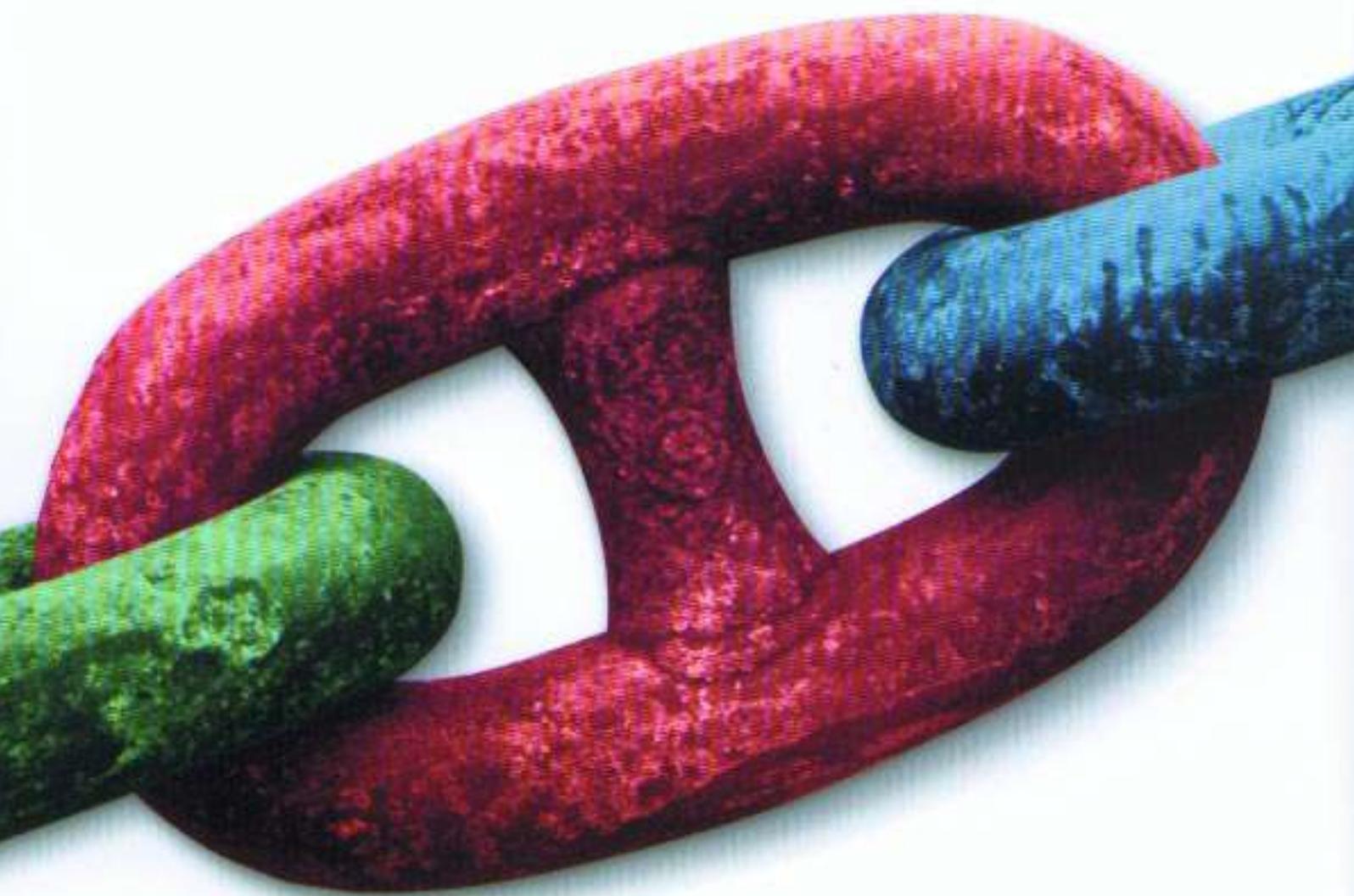
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*L'anello mancante
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