

Characterization, Classification, and Treatment of von Willebrand Diseases: A Critical Appraisal of the Literature and Personal Experiences

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ABSTRACT

Recessive type 3 von Willebrand disease (vWD) is a severe hemophilia-like bleeding disorder caused by homozygosity or double heterozygosity for two nonsense mutations (null alleles) and characterized by a strongly prolonged bleeding time (BT), absence of ristocetin-induced platelet aggregation (RIPA), absence of von Willebrand factor (vWF) protein, and prolonged activated partial thromboplastin time (APTT) due to factor VIII (FVIII:C) deficiency. Recessive severe type 1 vWD is caused by homozygosity or double heterozygosity for a missense mutation and differs from type 3 vWD by the detectable presence vWF:antigen (Ag) and FVIII:C levels between 0.09 and 0.40 U/mL. Carriers of one null allele or missense mutations are usually asymptomatic at vWF levels of 50% of normal. Mild recessive type 1 vWD may be due to a missense mutations, or one missense mutation plus blood group O. The so-called dominant type 1 vWD secretion defect and type 1 Vicenza are caused by a heterozygous missense mutation in the vWF gene that produces a mutant vWF protein having a dominant effect on the normal vWF protein produced by the normal vWF allele with regard to the defective processing, storage secretion, and/or proteolysis of vWF in endothelial cells and clearing from plasma consistent with a type 2 phenotype of vWD. Typical type 2 vWD patients, except 2N, show a defective vWF protein, decreased ratios for vWF:ristocetin cofactor [vWF:RCo]/vWF:Ag and vWF:collagen binding factor [vWF:CB]/vWF:Ag and prolonged BT. The BT is normal and FVIII:C levels clearly are lower than vWF:Ag in type 2N vWD. Multimeric analysis of vWF in plasma demonstrates that proteolysis of vWF is increased in type 2A and 2B vWD, with increased triplet structure of each band (not present in types 2M and 2U). Proteolysis of vWF is minimal in type 2C, 2D, and 2E variants that show aberrant multimeric structure of individual oligomers. vWD 2B differs from 2A by normal vWF in platelets, and increased RIPA. RIPA is normal in mild, decreased in moderate, and

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absent in severe type 2A vWD. RIPA is decreased or absent in 2M, 2U, 2C, and 2D; variable in 2E; and normal in 2N and dominant type 1. vWD 2M is usually mild and features decreased vWF:RCo and RIPA, and a normal or near-normal vWF multimeric pattern in a low-resolution agarose gel. vWD 2A-like or unclassifiable (2U) is distinct from 2A and 2B and typically features low vWF:RCo and RIPA with the relative lack of large vWF multimers. vWD type 2C is recessive; the dominant type 2D is rare. The response to desmopressin acetate (DDAVP) of vWF parameters is normal in pseudo-vWD and mild type 1. The responses to DDAVP of FVIII:C and vWF parameters in vWD 2M, Vincenza, 2E, and mild 2A, 2U, and 2N are transiently good for a variable number of hours to arrest mucocutaneous bleeding episodes or to prevent bleeding during minor surgery or trauma. However, the responses are not good enough to treat major bleedings or to prevent bleeding during major surgery or trauma. The response to DDAVP of vWF parameters is poor in recessive type 3, 1 and 2C, and dominant 2A, 2B, and 2U. Proper recommendations of FVIII/vWF concentrates using FVIII:C and vWF:RCo unit dosing for the prophylaxis and treatment of bleeding episodes in type 2 disease that is non-responsive to DDAVP and in type 3 vWD are proposed.

KEYWORDS: von Willebrand factor, von Willebrand disease, ristocetin cofactor activity, von Willebrand collagen binding activity, factor VIII:C, bleeding time, desmopressin acetate

The von Willebrand factor (vWF) is a multimeric plasma glycoprotein that plays a central role in hemostasis. vWF acts both as carrier for coagulation factor VIII (FVIII) in the plasma and as a mediator of platelet adhesion to subendothelium after vascular injury. Several distinct functional domains have been identified within the vWF, including regions involved in binding to factor VIII, to platelet receptor GPIb, to platelet GPIIb-IIIa, to components of extracellular matrix such as collagen and heparin, regions involved in multimerization and dimerization of vWF, and the A1 and A2 domains involved in the majority of type 2 von Willebrand disease (vWD) as recommended by the von Willebrand Factor Scientific Standardisation Committee (vWF-SSC) of the International Society on Thrombosis and Haemostasis (ISTH) is based on laboratory phenotyping using the combination of FVIII:C and vWF:antigen (Ag) levels, ristocetin-induced platelet aggregation (RIPA), and rather insensitive tests for vWF:ristocetin cofactor (RCo) and vWF multimeric pattern in a low-resolution agarose gel.^{2,3} Accumulating data on the structure and function relationship between phenotype and genotype of vWD and the use of more specific and sensitive diagnostic tools including vWF:collagen binding factor (CB) assay, FVIII binding to vWF, and improved multimeric analysis will have a major impact on a correct diagnosis and proper classification of the vWDs. The contribution of a DDAVP challenge test has become available for clinicians to better distinguish the various type 1 and type 2 vWDs. In this study, we evaluated the clinical features,

laboratory phenotypes, and genotypes of severe autosomal recessive type 3 and type 1 vWD, asymptomatic carriers of a nonsense or missense mutation in the vWF gene, blood group O-related vWF deficiency and mild or moderate dominant type 1 vWD, and all variants of type 2A, 2B, 2C, 2D, 2E, 2M, 2N, and 2 unclassifiable (U).

RECESSIVE TYPE 3 vWD AND BLOOD GROUP O

Obligate heterozygous parents of type 3 vWF patients in fact can be regarded as true type 1 quantitative vWF deficiency heterozygous for the vWF null allele. The inheritance of vWD type 3 is autosomal recessive.⁴⁻⁶ Type 3 vWD patients typically have strongly prolonged bleeding times (BT) and activated partial thromboplastin times; FVIII:C levels between 1 and 9%; undetectable vWF:Ag, vWF:RCo, and vWF:CB levels; and absence of RIPA.⁴⁻⁸ In 31 cases with type 3 vWD (age 2 to 80 years; median, 15 years) described by Schneppenheim et al,⁵ bleeding manifestations were recorded as easy bruising and prolonged epistaxis in 31 (100%), spontaneous joint bleedings in 23 (76%), muscle bleedings in seven (22%), and gastrointestinal bleedings in three (10%) patients. Type 3 vWDs are homozygous or compound heterozygous for two null alleles (gene deletions, stop codons, frameshift mutations, splice site mutations, and absence of mRNA) in the vast majority of reported cases.⁴⁻⁶ Compound heterozygosity for a null allele and a missense mutation or homozygosity for a missense mutation is rare in type 3 vWD.⁵

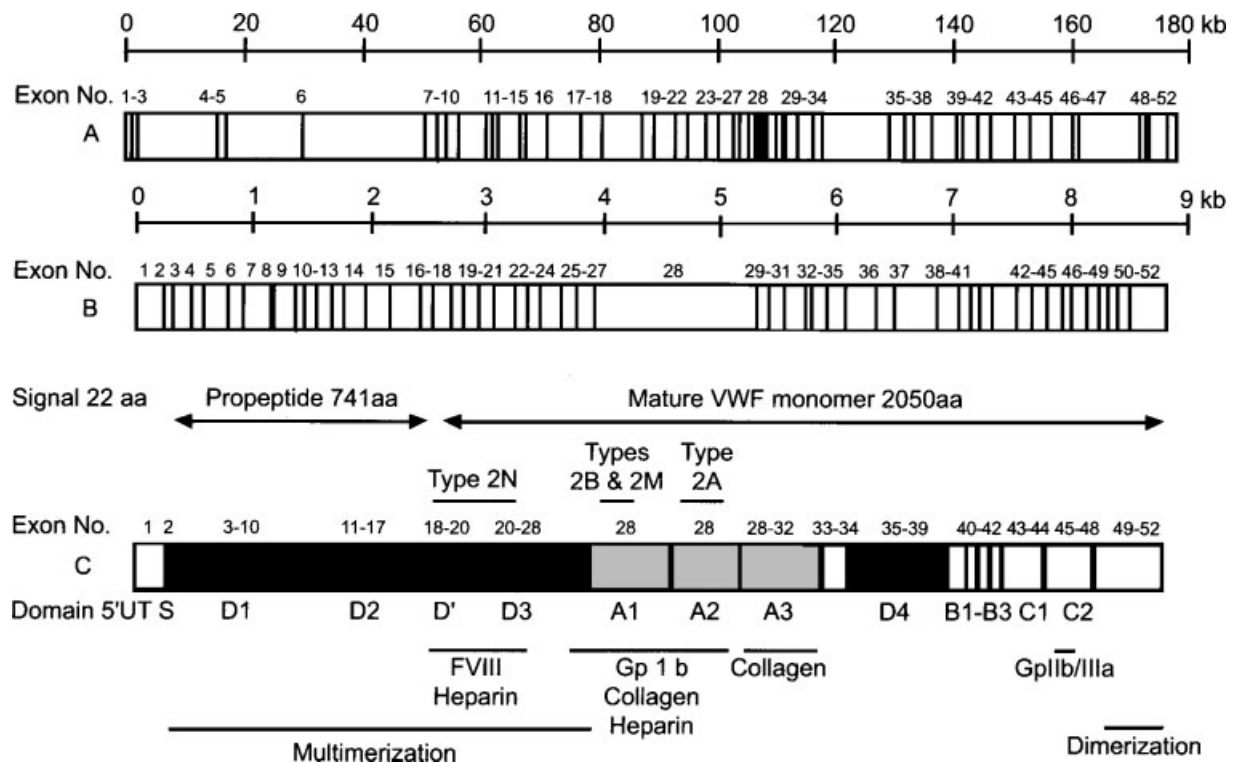


Figure 1 Structure and function relationship of the von Willebrand factor (vWF) gene and protein. For explanation, see text.

ASYMPTOMATIC HETEROZYGOUS TYPE 1 vWD CARRIERS AND BLOOD GROUP O

In the study of 27 patients with congenital type 3 vWF deficiency associated with one null allele analyzed by Schneppenheim et al,⁵ 22 were asymptomatic and only seven presented very mild bleeding (mainly bruising and epistaxis). All, except one, had normal BT. The mean values for FVIII:C, vWF:Ag, and vWF:RC₀ were 0.76, 0.39, and 0.39 U/mL, respectively, with an increased FVIII:C/vWF:Ag ratio of 1.9 and a normal vWF:RC₀/vWF:Ag ratio of 1, consistent with true type 1 vWF deficiency (Table 1). In the study of Zhang et al⁴ including 25 patients heterozygous for the vWF null allele and blood group non-O, 12 had no bleeding history and 13 presented with very mild bleedings (one or two bleeding symptoms, mainly epistaxis, bruises, and/or prolonged menstruations with no abnormal bleeding after tooth extraction). The mean values for FVIII:C and vWF:Ag were 0.81 and 0.45, respectively, with an increased ratio for FVIII:C/vWF:Ag of 1.8 (Table 1). In the same study of Zhang et al⁴ including 17 patients heterozygous for the vWF null allele and blood group A, eight had no bleeding history and 11 presented with minor bleedings. The mean values for FVIII:C and vWF:Ag were 0.74 and 0.32, respectively, with an increased ratio for FVIII:C/vWF:Ag of 2.3 (Table 1). In each of these two studies,^{4,8} there is a wide range of values from 0.11 to 1.28 U/mL for

FVIII:C and from 12 to 0.94 for vWF:Ag, with ratios of FVIII:C/vWF:Ag ranging from normal to increased above 2, indicating the difficulty in distinguishing true congenital type 1 vWF deficiency from vWF deficiency related to blood group O. In the study of Schneppenheim,⁵ the vWF parameters in the seven mildly symptomatic cases heterozygous for the vWF null allele ranged from 0.22 to 0.45 U/mL for vWF:Ag and from 0.24 to 0.48 for vWF:RC₀. In the study of Zhang,⁴ 24 individuals heterozygous for the vWF null allele (true type 1 vWF deficiency) had vWF:Ag levels above 0.50 U/mL in six and ranged from 0.13 to 0.45 U/mL in 18 patients. From these data it can be concluded that one fourth of the individuals with true vWF deficiency type 1 heterozygous for the vWF null allele are at risk for very mild bleedings at vWF values between 0.20 to 0.50 U/mL, do have normal BT, and are predicted to have a completely normal response of FVIII:C and vWF parameters from below 0.50 to above 1.00 U/mL before and after DDAVP.

RECESSIVE TYPE 1 vWD

Compound heterozygosity for a null allele and a missense mutation or homozygosity for a missense mutation is rare in type 3 vWD,⁴⁻⁶ but is common and traced frequently in patients with severe autosomal recessive type 1 vWD.⁸ Severe autosomal recessive type 1 vWD patients who are compound heterozygous for a null allele

Table 1 Laboratory Phenotype and Clinical Symptoms in 69 Patients with True vWF Deficiency Type 1 Heterozygous for the vWF Null Allele (parents of type 3 vWD)

Author	Schneppenheim ⁵	Zhang ⁴		Eikenboom ⁸	
Number of patients	27	25	17	14	6
Blood group	Not specified	A	O	A	O
FVIII:C (%)	76	81	74	93	81
Range	35–118	37–121	11–128	69–138	58–93
vWF:Ag	39	45	32	61	52
Range	27–68	13–94	12–70	37–98	40–66
vWF:RCo	39	—	—	56	53
Range	16–57	—	—	30–92	39–68
Ratio FVIII:C/vWF:Ag	1.9	1.8	2.3	1.52	1.56
Ratio vWF:RCo/Ag	1	—	—	1.66	1.52
Range	0.55–1.92				
Mild bleedings*	7	13	11	1	1
%	26%	52%	65%	7%	17%

*One or two bleeding symptoms mainly epistaxis, bruises and/or prolonged menstruation. No abnormal bleeding after tooth extraction or surgery, and no hemarthroses or muscle bleeding.

vWF, von Willebrand factor; vWD, von Willebrand disease; FVIII, factor VIII; Ag, antigen; RCo, ristocetin cofactor; C, coagulant.

and a missense mutation, or homozygous or double heterozygous for a missense mutation have detectable but very low vWF levels (Table 2).

Homozygotes for the missense mutations W377C (Schneppenheim et al⁵) and for R273W (Allen et al⁹) in the propeptide D1 domain have been described to be associated with severe autosomal recessive type 1 or type 3 vWD phenotype. Reported cases of severe

recessive type 1 vWD are in fact severe autosomal recessive type 2 vWD as demonstrated by vWF multimer analysis.^{9–13} The multimeric pattern of homozygous R273W clearly showed the absence of high molecular weight multimers and a pronounced monomeric band consistent with type 2A or 2E vWD.⁹ Homozygosity for the missense mutation C2364F in a family and double heterozygosity for C2364F/null in three families has

Table 2 Reports of Autosomal Recessive Severe Type 1 vWD Caused by Homozygous Missense Mutations or Compound Missense/Null Mutations with a Type 2 vWD on Multimeric Analysis of vWF Protein

Author/Mutation	F/M, Age	BT	FVIII:C	vWF:Ag	vWF:RCo	vWF:CB	RIPA	vWF:MM
Schneppenheim et al, 1994 ⁵								
W377C/W377C	2 yr	> 20	0.02	0.03	0.03	—	—	nt
Eikenboom et al, 1998, ⁸ Castaman et al, 2000 ¹¹								
C2362F/C2362F	F	> 30	0.19/0.44	0.02	< 0.03	—	—	2A
C2362F/splice site	M	> 30	0.01/0.28	0.01	< 0.03	—	—	2A
C2362F/R2535*nul	F	> 30	0.11/0.30	0.01	< 0.03	—	—	2A
C2362F/737insC	F	> 30	0.08/0.21	0.02	> 0.03	—	—	2A
C2671Y/del nul	F	> 30	0.10/0.19	0.03	< 0.03	—	—	2A
4699/?	M	15 > 20	0.25/0.37	0.11	< 0.03	—	—	
C2671Y/W2193R	M	> 30	0.18/0.33	0.6	< 0.03	—	—	
(Gene conversion)								
Castaman et al, 2002 ¹²								
C2362F/splice sit	M 3 yr	> 15	0.18	0.05	< 0.03	—	—	N low resolution
			0.21	0.07	0.07			
Schneppenheim et al, 2001 ¹³								
C2754W/C2754W		> 20	0.12	< 0.05	< 0.05	—	—	2D
Allen et al, 2000 ⁹								
R273W/R273W	Boy	15	0.20	0.06	0.06	—	—	2A or 2E
R273W/R273W	Boy	15	0.33	0.09	0.04	—	—	2A or 2E
R273W/R273W	Boy	> 20	0.09	< 0.01	< 0.01	—	—	—

vWD, von Willebrand disease; vWF, von Willebrand factor; FVIII, factor VIII; C, coagulant; Ag, antigen; RCo, ristocetin cofactor; CB, collagen-binding factor; RIPA, ristocetin-induced platelet aggregation; MM, missense mutation; nt, not tested.

been reported to be associated with severe type 1 vWD indicated by FVIII:C levels of 12 to 32 U/L, very low but detectable vWF:Ag, and undetectable vWF:RCo (Table 2).⁸ In some of these severe type 1 vWD patients, FVIII:C, vWF:Ag, and vWF:RCo reached values of >0.50, 0.11, and 0.09 U/L, respectively, after DDAVP.¹⁰ C2364F heterozygous carriers were asymptomatic, had normal or slightly prolonged BT, subnormal values for vWF:Ag and vWF:RCo with a normal vWF:RCo/Ag ratio, and a normal vWF multimeric pattern in a low 0.8 or 0.9% agarose resolution gel.¹¹ However, analysis of vWF in plasma from cases with severe autosomal recessive vWD homozygous for a missense mutation C2362F or compound C2362F/null (exon 42 of the B1 to B3 domain) as well as heterozygous carrier of C2364F all showed a heightened proteolytic pattern with marked increase of 176- and 140-kd degradation products consistent with type 2A vWD (Table 2).¹¹ Castaman et al¹² described another case of severe autosomal recessive type 1 vWD double heterozygous for two missense mutations C2364/splice site (Table 2).

Homozygosity for a dimerization defect in the C-terminal domain of the vWF gene C2754W caused type 3 vWD with undetectable vWF:Ag, vWF:RCo, and vWF:CB (Schneppenheim et al).¹³ Repeated vWF multimeric analysis of the patient's platelets could only detect low molecular weight multimers and further analysis revealed intervening bands between individual bands consistent with type 2D vWD. Eikenboom et al⁸ described a case of autosomal recessive type 1 vWD due to double heterozygosity for the mutation C2671Y in exon 49 of the dimerization region of the vWF gene for which analysis of the vWF multimeric pattern was not performed. Finally, clinicians have to be aware that type 2N vWD may present with normal BT and equally decreased vWF:Ag and vWF:RCo simulating recessive type 1 vWD, but FVIII:C is much lower compared with vWF:Ag due to a FVIII binding defect in the D' domain of the vWF protein.¹⁴

BLOOD GROUP O AND TYPE 1 vWD

ABO blood group is a well-known significant determinant of plasma vWF concentration.¹⁵⁻¹⁷ Coughlan et al¹⁸ evaluated the presence of vWF null alleles in a cohort of 36 unrelated type 1 vWD patients (according to SSC recommendations of the ISTH) and in a group of 82 control persons. vWF null alleles were not found in their type 1 vWD and also not in the control population. The frequency of blood group O in their type 1 patients was 72%, which is higher than the 43% in their control population. Of 62 parents of type 3 patients with a documented null allele, 23 (37%) had blood group O (Table 1). These observations reflect the complexity underlying the expression of a laboratory phenotype

type 1 vWD, and suggest the involvement of asymptomatic heterozygous missense mutations in the vWF gene and genetically determined factors such as blood group O. It is unknown whether additional congenital or acquired factors also exist. This view is supported by the observation that the missense mutation (polymorphism) Y1584C in exon 26 of the vWF gene is increased in symptomatic vWD type 1 (14%) and the majority of these Y1584C heterozygotes (approximately 90%) were of blood group O.^{19,20} Bleeding manifestation in probands in nine type 1 families with the Y1584 mutation and blood group O were mild in two families and mild to moderate in seven families.¹⁹ The laboratory phenotype of probands with the combination of Y1584C and blood group O was typically type 1 vWD, with mean values of FVIII:C 0.54 (0.11 to 1.01), vWF:Ag 0.40 (0.24 to 0.50), and vWF:RCo 0.36 (0.29 to 0.46).¹⁹ Heterozygosity for the missense mutation Y1584C was found in two of 200 random controls by Bowen et al,²⁰ and in one asymptomatic case (vWF:Ag 0.50 U/mL) among 100 healthy controls by O'Brien.¹⁹ In a subsequent study Bowen et al²¹ studied 30 type 1 vWD families and found that heterozygosity for Y1584C was present in eight of 30 (27%) families and 19 of 76 (25%) individuals with mild type 1 vWD. Eighteen (95%) of these heterozygous (C1584) individuals had blood group O. These data confirmed previous observations that C1584 associated with blood group O is prevalent among mild type 1 vWD, but not necessarily causative of disease in all cases. C1584 does not by itself cause type 1 vWD. Increased vWF-cleaving metalloprotease ADAMTS 13-induced proteolysis of vWF was associated with C1584 and cosegregated with C1584 in affected and nonaffected individuals.²¹ Therefore, C1584 should not be used in isolation to diagnose mild type 1 vWD.

PSEUDO-vWD

We prospectively studied the probands of 24 unrelated families diagnosed with so-called mild vWD type 1.²² Bleeding manifestations were mild and the BT usually normal on repeated occasions. The levels for vWF:Ag, vWF:RCo, and vWF:CB were between 0.20 to 0.60 U/mL, with normal ratios for vWF:RCo/vWF:Ag.²² FVIII:C levels were somewhat higher compared with vWF:Ag, with a FVIII:C/vWF:Ag ratio of 1.48 (range, 0.8 to 2.2). vWF:Ag levels of 0.20 and 0.60 before DDAVP reached high peak values of above 1.0 U/mL in all with a mean of 2.16 U/mL (range 1.15 to 3.18 U/mL) after DDAVP.²² After DDAVP the ratios of vWF:RCo/vWF:Ag remained normal (0.97, range 0.65-1.34), but the ratio of vWF:CB/vWF:Ag increased significantly to 1.60 (range 1.03-2.28) indicating the release of unusual high vWF multimers immediately after desmopressin acetate

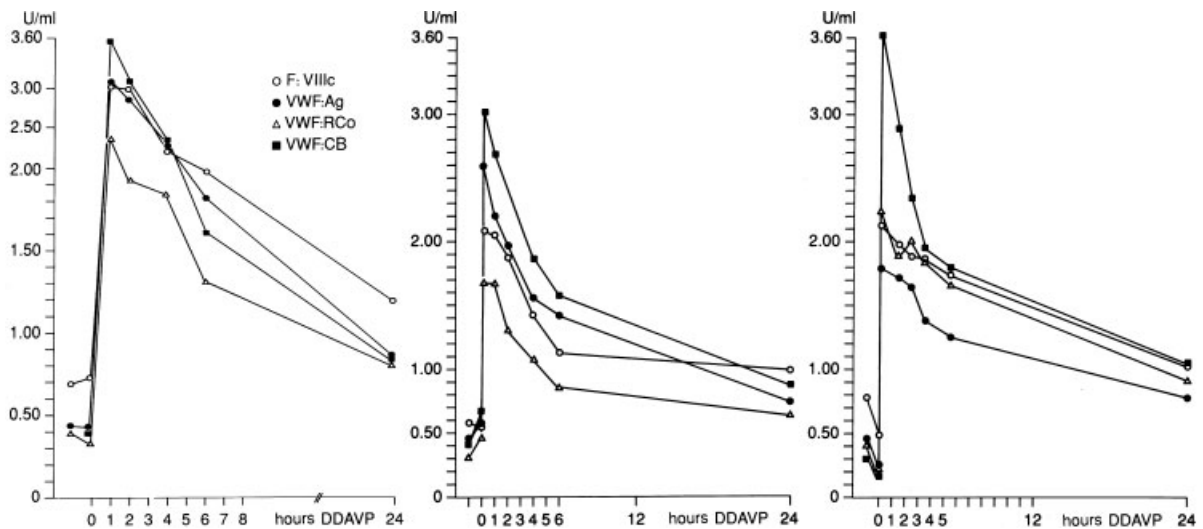


Figure 2 Normal responses to desmopressin acetate (DDAVP) of factor VIII coagulant activity (FVIIIc) and von Willebrand factor (vWF) parameters in three patients with mild vWF deficiency consistent with pseudo-von Willebrand disease (vWD).

(DDAVP; Fig. 2).²² Interestingly, the ratio FVIII:C/vWF:Ag remained normal or corrected to normal after DDAVP in nearly all probands (Fig. 2), which is suggestive of a pseudo-vWF deficiency rather than a

genetically determined true type 1 vWF deficiency.²² The values for FVIII:C and vWF parameters remained in the low normal range for 24 hours after DDAVP infusion (Fig. 2).

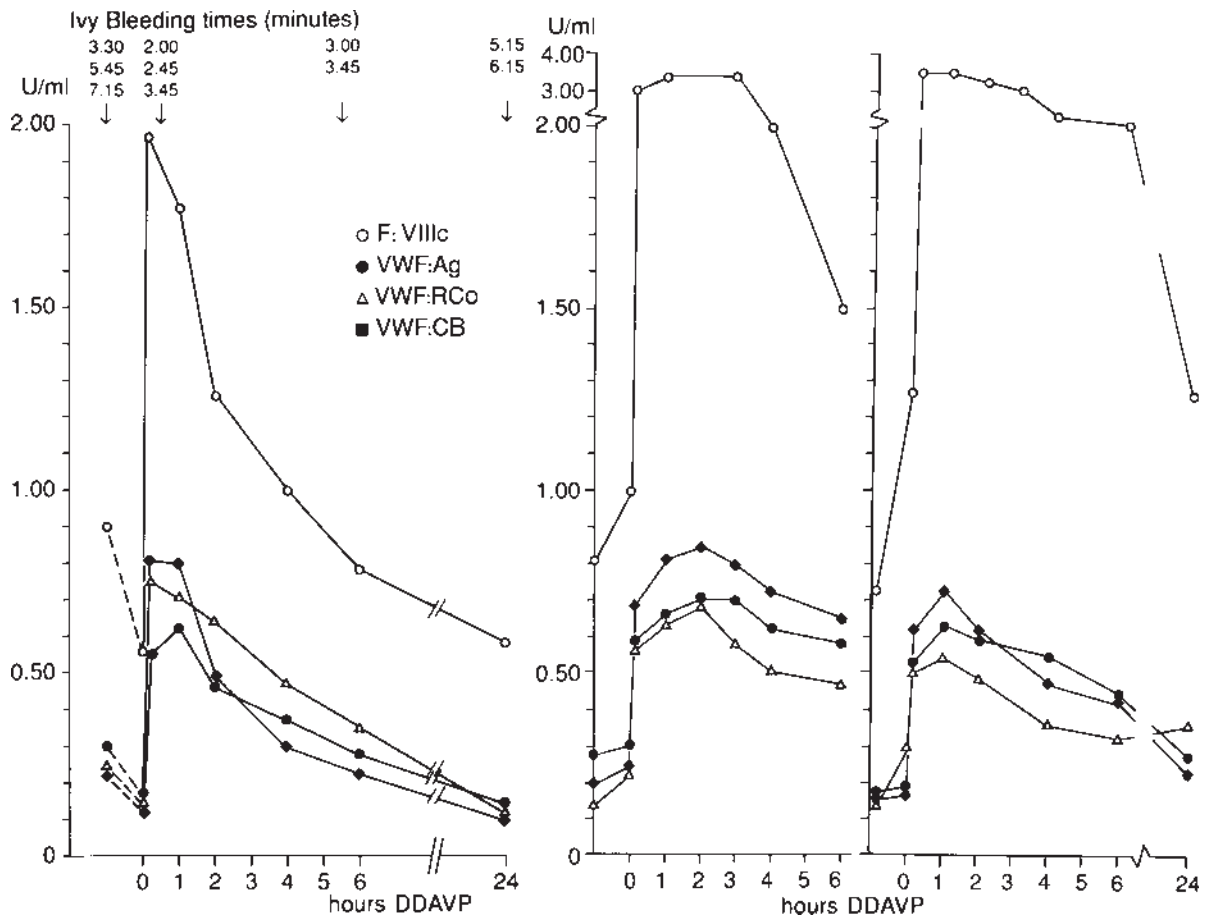


Figure 3 Normal response of factor VIII coagulant activity (FVIIIc) and decreased response of von Willebrand factor (vWF) parameters to desmopressin acetate (DDAVP) in three cases of dominant type 1 von Willebrand disease (vWD); one woman, left, and one brother and sister, middle and right, respectively showing an increased ratio of FVIII:C/vWF:antigen (Ag) and a normal vWF:ristocetin cofactor (RCO)/Ag ratio before and after DDAVP consistent with a secretion defect.

Table 3 Laboratory Data in Six Families with Autosomal Dominant Type 1 vWD

Author	BT	FVIII:C (U/dL)	vWF:Ag (U/dL)	vWF:RCo (U/dL)	vWF:CB (U/dL)	Ratio vWF:CB/Ag	Ratio FVIII:C/ vWF:Ag	RIPA	Response to DDAVP	vWF:MM
Michiels et al, 2002 ²²										
Family 1	Normal	19	9	< 10	12	1.3	2.1	N	Decreased type 1	N
Family 2	↑	56	17	16	13	0.8	3.3	N	Decreased type 1	N
Family 3										
Mother	↑	67	19	14	21	1.1	3.5	N	Decreased type 1	N
Daughter	↑	65	19	23	16	0.9	3.4	N	Decreased type 1	N
Brother	↑	82	21	17	27	1.3	3.9	N	Decreased type 1	N
Eikenboom et al, 1996 ²⁴										
Family 4										
Father	↑	21	10	< 20	—	—	2.1	—	—	N
Son	↑	22	11	< 20	—	—	2.0	—	—	N
Daughter	↑	35	15	< 20	—	—	2.3	—	—	N
Federici et al, 1993 ²⁹										
Grandfather	↑	35	10	7	—	0.7	3.5	—	—	N
Mother	↑	28	8	6	—	0.75	3.5	—	Decreased	N
Daughter	↑	24	10	6	—	0.60	2.4	—	—	N

vWD, von Willebrand disease; BT, bleeding time; vWF, von Willebrand factor; FVIII, factor VIII; C, coagulant; Ag, antigen; RCo, ristocetin cofactor; CB, collagen-binding factor; RIPA, ristocetin-induced platelet aggregation; DDAVP, desmopressin acetate; MM, missense mutation; ↑, prolonged; —, not tested; N, normal vWF multimers in low-resolution agarose gel.

DOMINANT TYPE 1 vWD SECRETION DEFECT

In our cohort of vWD patients,²² we found three families with severe type I vWD (vWF parameters < 0.20 U/mL), who had (1) a mild to moderate bleeding history since childhood, (2) a normal RIPA, (3) a normal or slightly prolonged Ivy BT, and (4) a normal vWF multimeric pattern but a decreased response of vWF parameters to DDAVP, suggesting a secretion defect of vWF from endothelial cells (Fig. 3). In these three probands, the ratio of FVIII:C/vWF:Ag was > 3 before and after DDAVP (Table 3), which is suggestive of a decreased synthesis or secretion defect due to an undefined vWF missense mutation. We propose to label this category as autosomal dominant moderate vWD type 1 due to a secretion defect (Table 3).²³

Eikenboom et al²⁴ demonstrated that the classical autosomal dominant type 1 vWD with high penetration of moderate bleeding symptoms is caused by a heterozygous missense mutation Cys³⁶⁷ → Phe (C1130F) in one and Cys³⁸⁶ → Arg (C1149R) in another family. These two missense mutations are located in the D3' domain and interfere with the normal vWF subunits coded by the normal vWF allele, causing a defective intracellular multimerization and degradation of vWF, leading to a secretion defect of the vWF by endothelial cells.^{24,25} The autosomal dominant severe type 1 vWD (C1130F) is characterized by low vWF levels of < 0.20 U/mL and the presence of all sizes of vWF multimers, and an increased FVIII:C/vWF:Ag ratio of 2.0 to 2.3 consistent with a synthesis → secretion defect

of vWF deficiency type 1 (Table 3).²³ A similar dominant-negative mechanism of intracellular retention and degradation of vWF caused by heterodimerization of mutant and normal vWF subunits in the endoplasmic reticulum followed by proteosomal degradation in the cytoplasm has been described for the C1149R mutation. The laboratory features of this autosomal dominant type 1 mutant are not reported.²⁵ Castaman et al²⁶ screened 24 unrelated Italian patients with autosomal dominant type 1 vWD for the C1149R and C1130F mutations. None of the patients had the C1149R mutation and three apparently unrelated patients showed the presence of the C1130F mutation (formerly reported as C367F) and typical features of severe dominant type 1 vWD, with vWF values < 15 U/dL and increased FVIII:C/vWF:Ag ratios.²⁶ The C1130F and C1149R mutations indeed showed impaired multimerization by the absence of high molecular weight multimers; they also showed a pronounced secretion defect caused by intracellular retention and degradation of mutant vWF, consistent with a quantitative vWD phenotype.²⁴⁻²⁷ Such cases of autosomal dominant type 1 vWD with type 2-like vWF multimeric pattern due to cysteine mutations in the D3 domain (C1130F and C1149R) are very likely to be reclassified as type 2E vWD (see 2E vWD section).

Casana et al²⁸ reported the association of dominant mild type 1 vWD with increased FVIII:C/vWF:Ag ratios in seven members of one family with heterozygosity of T1156M in the D3 domain of the vWF gene. Federici et al²⁹ described a family (grandfather, mother, and newborn daughter) with severe type 1

vWD, subtype plasma low-platelet low that could be explained by decreased synthesis of vWF in cultured endothelial cells isolated from the umbilical vein of the newborn daughter. This family can readily be reclassified as autosomal dominant severe type 1 vWD (Table 3).

vWD TYPE 1 vWD VICENZA DIFFERS FROM TYPE 2M

In our cohort of vWD patients,²² we found two probands with severe type 1 vWD and decreased RIPA, that could be classified as vWD type 2M because of a very characteristic response to DDAVP (Fig. 4). The typical laboratory features in our vWD type 2M patients are (1) severe type 1, (2) decreased RIPA in the presence of a normal or near-normal vWF multimeric pattern in a low-resolution agarose gel, (3) a poor response of vWF:RCo to DDAVP, and (4) a good response of both vWF:CB and vWF:Ag to DDAVP. This good response of vWF:CB to DDAVP in 2M vWD is consistent with the presence of all vWF multimers and can explain the slightly prolonged and normal Ivy BTs before and after DDAVP (Fig. 4).²² In a recent study of 317 patients previously registered as type 1 vWD, 30 patients from 17 unrelated families with discrepant vWF:RCo/vWF:Ag ratios in plasma, normal vWF multimers could be reclassified as type 2M.³⁰ RIPA assay was previously performed in 26 of 30 2M patients and an absent or decreased responsiveness to ristocetin with minor aggregation at 1.5 mg/mL was found in all cases.³⁰ This simply may mean that all cases of severe type 1 vWD with a normal or near-normal vWF multimeric pattern and a decreased or absent RIPA, and a decreased vWF:RCo/vWF:Ag ratio, can currently be reclassified as 2M vWD.

Congenital vWD Vicenza clearly differs from 2M and 2U. vWD Vicenza is characterized by equally low levels of FVIII:C, vWF:Ag, and vWF:RCo; usually normal RIPA; a normal or slightly prolonged BT; and the presence of unusually large vWF multimers in plasma.^{17,31,32} The response to DDAVP in several cases of vWD Vicenza^{32,33} was good for FVIII:C, vWF:Ag, and vWF:RCo, which was followed by unexplained very short half-life times of less than a few hours for FVIII:C and all vWF parameters, consistent with a laboratory type 1 phenotype. The ratios for FVIII:C/vWF:Ag, vWF:RCo/vWF:Ag, and vWF:CB/vWF:Ag remained normal before and after DDAVP.³¹⁻³³ A single mutation R1205H in the D3 (multimerization) domain was found as the probable cause of vWD type Vicenza.³⁴

On the basis of this update and detailed analysis of the literature in a previous study,³⁵ we propose a novel classification and characterization of type 1 and type 3 vWD patients (Table 4). Carriers of recessive type 1 and type 3 vWD are in fact true type 1 vWD at the genetic level, but are usually asymptomatic or have mild bleed-

ing, particularly when associated with blood group O. Carriers of a missense mutation in the vWF gene may be asymptomatic (polymorphism), for example C1584, and may be mildly symptomatic in particular when associated with blood group O. Variants of dominant type 1 vWD result from a missense mutation in the vWF gene with dominant negative effect of the mutated vWF protein on the normal vWF protein causing a defective intracellular processing and secretion of vWF by endothelial cells and defective clearing of the secreted vWF from plasma consistent with a type 2E or type Vicenza vWD.

TYPE 2 vWD

In the plasma, vWF appears as a series of large and intermediate multimers of regularly decreasing molecular mass, from several thousand to 500 kd.^{36,37} The size of circulating vWF multimers is controlled by proteolytic cleavage performed by a specific protease, ADAMTS 13, at cleavage site 1506 in the A2 domain. The 140-kd fragment corresponds to residues 763 to 1605aa, and the 176-kd fragment to residue 1605 to 2813aa. The vWF is an adhesive protein that mediates the initiation of platelet adhesion and aggregation through vWF subendothelial and vWF-platelet interactions to control primary hemostasis (BT) at sites of vascular trauma or injury. vWF binds coagulant FVIII (FVIII:C), contributing to its stability and, indirectly, to its function in the generation of fibrin.³⁷

Using high-resolution sodium dodecyl sulfate-agarose multimeric analysis of vWF in plasma in combination with immunoblots of vWF proteolytic degradation products, Zimmerman et al³⁸ nicely demonstrated that proteolysis of vWF is a normal event in normal individuals, and is increased in types IIA (2A) and IIB (2B) vWD with increased triplet structure of each band as the result of proteolysis, and that proteolysis of vWF is minimal in types IIC (2C), IID (2D), and IIE (2E) variants with aberrant multimeric structure of individual oligomers.³⁸ In types IIA (2A) and IIB (2B), the proportion of 176- and 140-kd fragments was increased related to the intact 225-kd subunit, and these degraded vWF fragment were not detected or were present in only trace amount in types IIC (2C), IID (2D), and IIE (2E) vWD.^{38,39} Zimmerman et al³⁸ therefore postulated additional mechanisms to explain the aberrant structure of individual oligomers in types IIC, IID, and IIE vWD patients. All types IIA, B, C, D, and E^{2,3,35} are reclassified by Schneppenheim, Budde, and Ruggeri as 2A, 2B, 2C, 2D, and 2E (Fig. 5; Table 5)^{39,40}

DOMINANT TYPE 2A vWD

The pertinent findings in patients with type 2A vWD include prolonged BT; consistently low vWF:RCo/

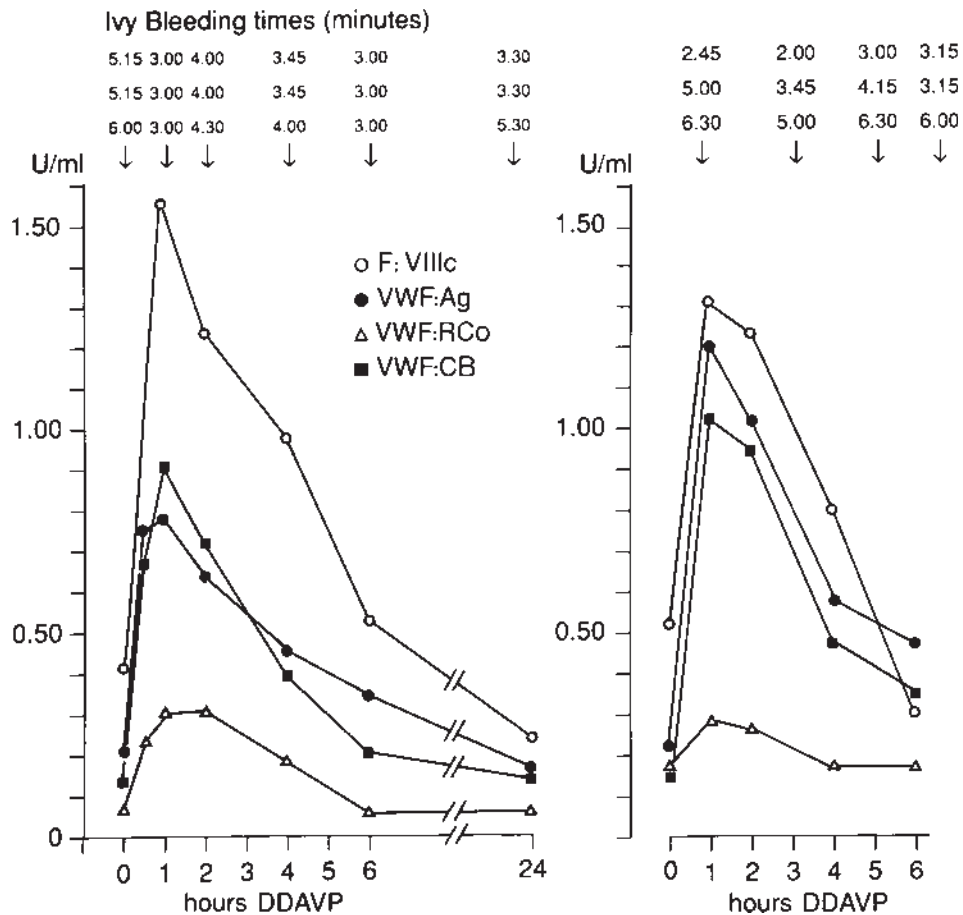


Figure 4 Poor response of von Willebrand factor (VWF):ristocetin cofactor (RCo) and good responses of factor VIII coagulant activity (FVIIIc), vWF:antigen (Ag), and vWF:collagen-binding factor (CB) to intravenous desmopressin acetate (DDAVP) in two cases with type 2M von Willebrand disease (vWD).

Table 4 The Antwerp Classification and Characterization of Quantitative Type 1 and Type 3 vWD

Category vWD	BT	FVIII:C (%)	vWF:Ag (%)	RCo (%)	RIPA	Bleeding Type	vWF Gene Mutation
Severe type 3, recessive	↑↑↑	1-9	Zero	Zero	Zero	Severe hemophilia	Double nonsense
Severe type 1, recessive	↑↑↑	9-40	1-10	0-6	Zero	Moderate severe	Double missense
Bloodgroup O^{15,16,17} (pseudo-vWD)	N	35-150	35-150	35-150	N	Asymptomatic very mild	Normal vWF gene
Carrier type 3 Minor influence (-10%) of blood group O	N/↑	30-140	15-90	15-90	N	Asymptomatic very mild	Single nonsense (null allele)
Carrier type 1 (polymorphism)	N	N	N	N	N	Asymptomatic	Single missense
Mild type 1	N/↑	12-70	14-70	12-80	N	Mild	Y1584C ¹⁹⁻²¹ /blood group O
Variable penetrance							(multigenetic)
Dominant type 1 Secretion defect	N/↑	20-80	10-40	0-30	N	Mild or Moderate	Single missense D3 domain type 2E
Dominant type 1 Vicenza	N/↑	< 15	< 15	< 15		Moderate	Single ^{R1205H} missense (variant 2M)

vWD, von Willebrand disease; BT, bleeding time; FVIII, factor VIII; C, coagulant; Ag, antigen; RCo, ristocetin cofactor; RIPA, ristocetin-induced platelet aggregation; vWF, von Willebrand factor.

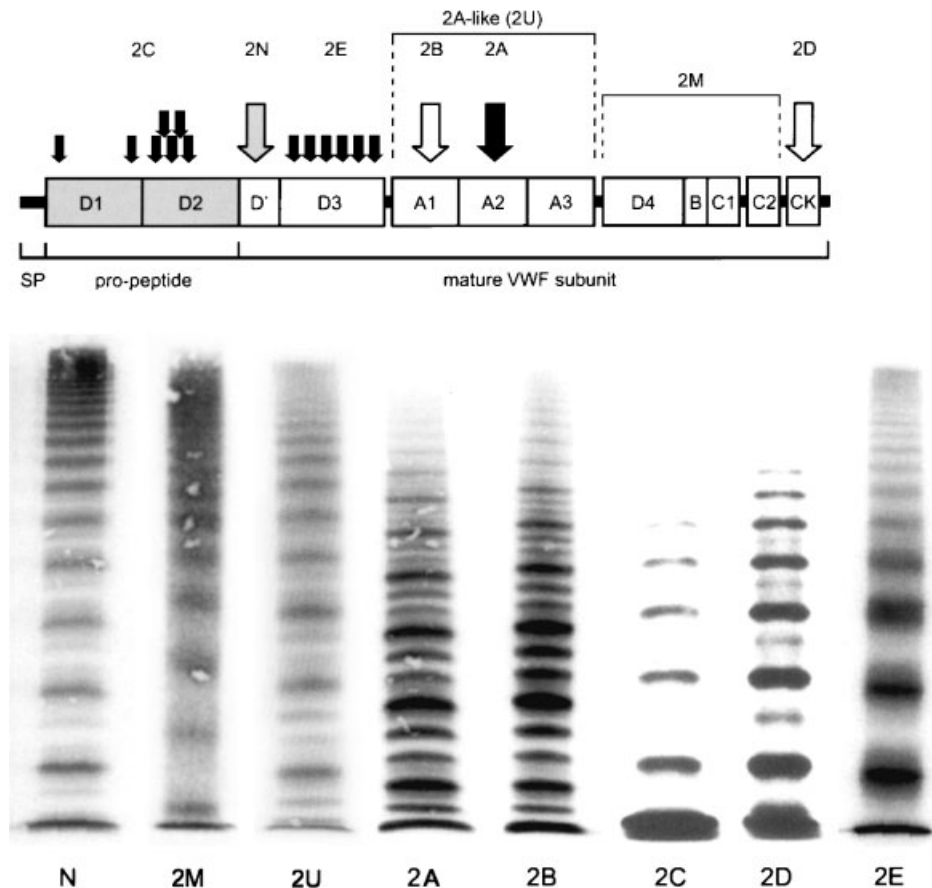


Figure 5 Clustering of von Willebrand factor (vWF) mutations for each of the variants of type 2 von Willebrand disease (vWD) to specific domains of the vWF gene and protein and the typical multimeric pattern characteristic for each variant of type 2 vWD according to Budde and Schneppenheim.⁴⁰

vWF:Ag and vWF:CB/vWF:Ag ratios; absence of high, and depending on severity, absence of intermediate vWF multimers; pronounced triplet structure of individual bands; and increased vWF degradation products (Fig. 5; Table 5).³⁸⁻⁴⁰ Type 2A vWD results from genetic abnormalities in the A2 domain of the vWF gene, and the absence of high vWF multimers and increased triplet structure is the consequence of increased *in vivo* proteolysis of large vWF multimers.⁴¹⁻⁴⁴ Lyons et al⁴⁵ demonstrated that structural changes within the A2 domain can produce two different characteristic phenotypes of type 2A vWD. In COS cells, three single missense mutations Val844Asp (V1607D), Ser743Leu (S1506L), and Gly742Arg (G1505R) resulted in no secretion of high molecular weight multimers due to impaired transport of vWF multimers between the endoplasmic reticulum and the Golgi complex (so-called group 1 defect) with very likely intracellular proteolysis of large vWF multimers.⁴⁵ The two missense mutations Arg834Trp (R1597Q) and Gly742Glu (G1505E) resulted in secretion of high molecular weight multimers similar to wild-type multimers, indicating that extracellular loss of high molecular weight multimers due to

increased proteolysis in plasma is responsible for type IIA (2A) vWD (so-called group 2 defect). Interestingly, platelet lysates demonstrated a decrease of large vWF multimers for G1505L and S1506L mutants (group 1 defect) but a normal pattern for the G1505E and R1597W mutants (group 2 defect).

Battle et al^{46,47} and Michiels et al²² observed heterogeneity of mild, moderate, and severe type 2A vWD with regard to bleeding symptoms, laboratory phenotypes, and response to DDAVP (Table 6). Mild type 2A vWD is characterized by normal or subnormal values for FVIII:C and vWF:Ag; low vWF:RC₀ values > 0.20; normal RIPA; a good but transient correction of BT, FVIII:C, and vWF parameters; and large multimers for a few hours after DDAVP (Fig. 6). Pronounced type 2A vWD patients have low values for vWF:Ag, very low or undetectable levels for vWF:RC₀ and vWF:CB, and no RIPA at a high concentration of ristocetin (1.75 or 2.0 mg/mL). The response of BT and the functional vWF parameters to DDAVP in mild and pronounced type 2 vWD patients (Table 6) is variable, ranging from transient for less than 3 hours, to minor or very poor (Fig. 6).

Table 5 Classification and Characterization of Type 2 vWD According to Zimmerman and Dent,³⁸ Schneppenheim et al,³⁹ and Budde and Schneppenheim⁴⁰

Type	Ratio vWF:RCo/Ag	vWF Multimeric Pattern (see figure 5)	Molecular Defect Domain/Mechanism	RIPA	Heredity
2A	Decreased	Absence of high vWF MM, pronounced triplet structure	A2 domain/increased proteolysis of vWF	N ↓/↓ ↓ or A	AD
2B	Decreased	Absence of high vWF MM, pronounced triplet structure	A1 domain/increased proteolysis of vWF	↑ ↑	AD
2C	Decreased	Absence of high vWF MM, absence of triplet structure	D1 D2 domain/multimerization defect	↓ ↓/A	AR
2D	Decreased	Absence of high vWF MM, absence of triplet structure and odd number of monomers	CK end of vWF gene/dimerization defect	↓	AD
2E	Decreased	Relative lack of high MM, triplet structure: absence of outer band and inner band closer to central band	D3/multimerization defect	N/↓	AD
2N	Normal	Normal, FVIII:C/vWF:Ag ratio decreased	D' FVIII binding domain	N	AR
Present and Previous Study According to Michiels et al⁵⁶					
Vicenza	Normal	Presence of unusually high vWF MM mimicking type 1	A1 domain	N	AD
2M	Decreased	The presence of high vWF MM mimicking type 1 but with a decrease or absence of subbands	A1 domain RIPA defect	↓ ↓/A	AD
2U	Decreased	Relative decrease of high vWF MM (1B variant 2A)	A1 domain RIPA defect	↓ ↓/A	AD
2E like	Decreased	Relative lack of high MM; triplet structure: absence of outer band and inner band closer to central band	D3 domain, no data?	N/↓/↓ ↓	AD or AR

vWD, von Willebrand disease; vWF, von Willebrand factor; RIPA, ristocetin-induced platelet aggregation; MM, missense mutation; AD, autosomal dominant; AR, autosomal recessive.

DOMINANT TYPE 2B vWD

The laboratory phenotype of type 2B is indistinguishable from type 2A. Types 2A and 2B are characterized by prolonged BT, consistently low vWF:RCo/vWF:Ag and vWF:CB/vWF:Ag ratios, absence of high and some of the intermediate vWF multimers with pronounced triplet structure of individual bands, and increased vWF degradation products (Fig. 5; Table 5),³⁸⁻⁴⁴ but 2B differs from 2A by a normal vWF multimeric pattern in platelets, and increased RIPA. For type 2B vWD, the response to DDAVP is good for FVIII:C and vWF:Ag, but despite the fact that vWF:RCo increases to >1.0 U/mL, the vWF:CB does not increase to normal levels, and there is no correction of BT and large vWF multimers after DDAVP.²² Patients with type 2B vWD may respond to an infusion with DDAVP with thrombocytopenia due to the appearance of higher vWF multimers, causing reversible platelet aggregation in vivo.

DOMINANT vWD TYPE 2M AND TYPE 2 U

The determination and characterization of type 2M vWD in the literature is problematic. The very first case labeled as vWD 2M (previously labeled as type 1B vWD^{48,49}) showed the mutation Gly561Ser (G1324S), a slight qualitative defect in multimer distribution, a completely normal vWF multimer distribution, normal values of FVIII:C and vWF:Ag, but absence of vWF:RCo and RIPA, a profound decrease of vWF binding to platelets in the presence of ristocetin but a normal binding of vWF to platelets in the presence of botrocetin.⁴⁸⁻⁵⁰ There was no genetic evidence for either a dominant or recessive inheritance because family members of the affected patient were not available, but circumstantial evidence was produced that Gly561Ser (G1324S) may be a recessive mutation and that the other allele may not be expressed (null allele).⁴⁸⁻⁵² This single observation is at the origin of the definition of type 2M

Table 6 Heterogeneity of Patients with Dominant Type 2A vWD

Case	Sex	BT Minutes	BT After DDAVP	MM After DDAVP	FVIII:C (U/mL)	vWF:Ag (U/mL)	RCo	CB	RIPA 1.2/2.0
Battle et al 1986, 1994 ^{46,47}									
Mild type 2A vWD									
1	F	> 20	4.5	—	0.35	0.46	0.22	—	+/+
2	M	> 20	7	—	0.30	0.50	0.22	—	+/+
3	F	15	8	TCC	0.96	1.52	0.7	—	-/+
Pronounced type 2A vWD									
4	F	> 30	9.5	TPC	1.00	0.50	< 0.01	—	-/+
5	F	> 30	16	NC	0.50	0.40	< 0.01	—	-/-
6	M	> 30	21	—	0.39	0.26	0.05	—	-/-
Normal					> 0.50	> 0.45	> 0.45		+/+
									RIPA
Michiels et al, 2002 ²²									
Mild type 2A vWD									
1	F	4–10	< 4	TCC	0.62	0.98	0.30	0.23	Normal
2	M	6–10	< 4	TCC	0.92	0.56	0.28	0.23	Normal
3	F	3–6	< 4	TCC	0.37	0.42	0.21	0.18	Normal
Pronounced type 2A vWD									
4	F	> 15	3–9	—	0.93	0.45	0.13	0.05	Decreased
5	F	> 15	4–8	NC	0.38	0.15	< 0.10	< 0.05	Absent
6	F	> 15	> 15	NC	0.66	0.41	0.15	0.05	Absent
Normal					> 0.60	> 0.60	> 0.60	> 0.60	Normal

vWD, von Willebrand disease; BT, bleeding time; DDAVP, desmopressin acetate; MM, missense mutation; FVIII, factor VIII; C, coagulant; Ag, antigen; RCo, ristocetin cofactor; CB, collagen-binding factor; RIPA, ristocetin-induced platelet aggregation; TCC, transient complete correction of vWF multimers; TPC, transient partial correction of vWF multimers; NC, no correction of vWF multimers.

vWD.^{2,3} Those patients with a decreased ristocetin-induced platelet-dependent function (vWF:RCo) in the presence of large vWF multimers are subsequently classified as 2M variants.² Severe type 1 “platelet discrepant” described by Mannucci et al (1985)⁵¹ has been reclassified as vWD 2M by Federici et al,⁵² and as type 2A by Sadler.²

Since 1996, 22 patients from eight unrelated families with a typical 2M phenotype of vWD have been described in the literature^{53–55} and reviewed in great detail.⁵⁶ Genotypes underlying the 2M vWD phenotype included the following defects in the A1 domain: G1324S, Q1191del11, F1369I, I1425F, K1408delK, Y1321D, R1394I, and one defect in the A2 domain I1526T. The 2M vWDs are characterized by near-normal values for FVIII:C (mean 53 U/dL), subnormal levels for vWF:Ag (mean 39 U/dL), and consistently very low levels for vWF:RCo between < 5 to 30 U/mL, with decreased vWF:RCo/vWF:Ag ratios between 0.40 and 0.12. RIPA was measured in 13 of the 22 type 2M patients and proved to be decreased uniformly. There is no consensus about the definition of normal high molecular weight multimers. Three of the 2M vWD patients showed a near normal but quantitative loss of the highest vWF multimers in two studies,^{54–56} whereas a normal vWF multimeric pattern

has been noted in the other 19 2M patients.^{53–56} RIPA was not measured in 14, but uniformly decreased or absence in 8, which constitutes a very characteristic feature of vWD 2M.^{53–56}

Since 1995, 106 patients from 12 unrelated families with the genetic defect R1374H (Arg611His) or R1374C (Arg611Cys) have been published^{55,57–59} and reviewed in great detail.⁵⁶ Lethagen et al⁵⁷ found the R1374C mutation in 72 patients from four families, which were diagnosed as severe type 1 vWD (mean value for vWF:Ag, 0.21; mean value for vWF:RCo, 0.13; ratio, 0.62). The values for vWF:Ag in 63 affected families ranged from 0.12 to 0.72, and the values for vWF:RCo in 33 affected family members ranged from 0.01 to 0.88. In this family, the vWF:RCo was often lower than the vWF:Ag, also indicating a qualitative defect of the vWF protein.⁵⁷ The high molecular weight vWF multimers were often more reduced than the low molecular weight multimers, and such cases have been classified as type 1B by Hoyer et al,⁵⁸ and as the 1B variant of 2A by Schneppenheim et al³⁹ (Fig. 5; Table 5). In the study of Castaman et al,⁵⁹ the R1374H mutation in two families was classified as type 1 platelet discordant, a subtype characterized by disproportional low vWF:RCo in plasma and platelets as compared with the vWF:Ag levels.

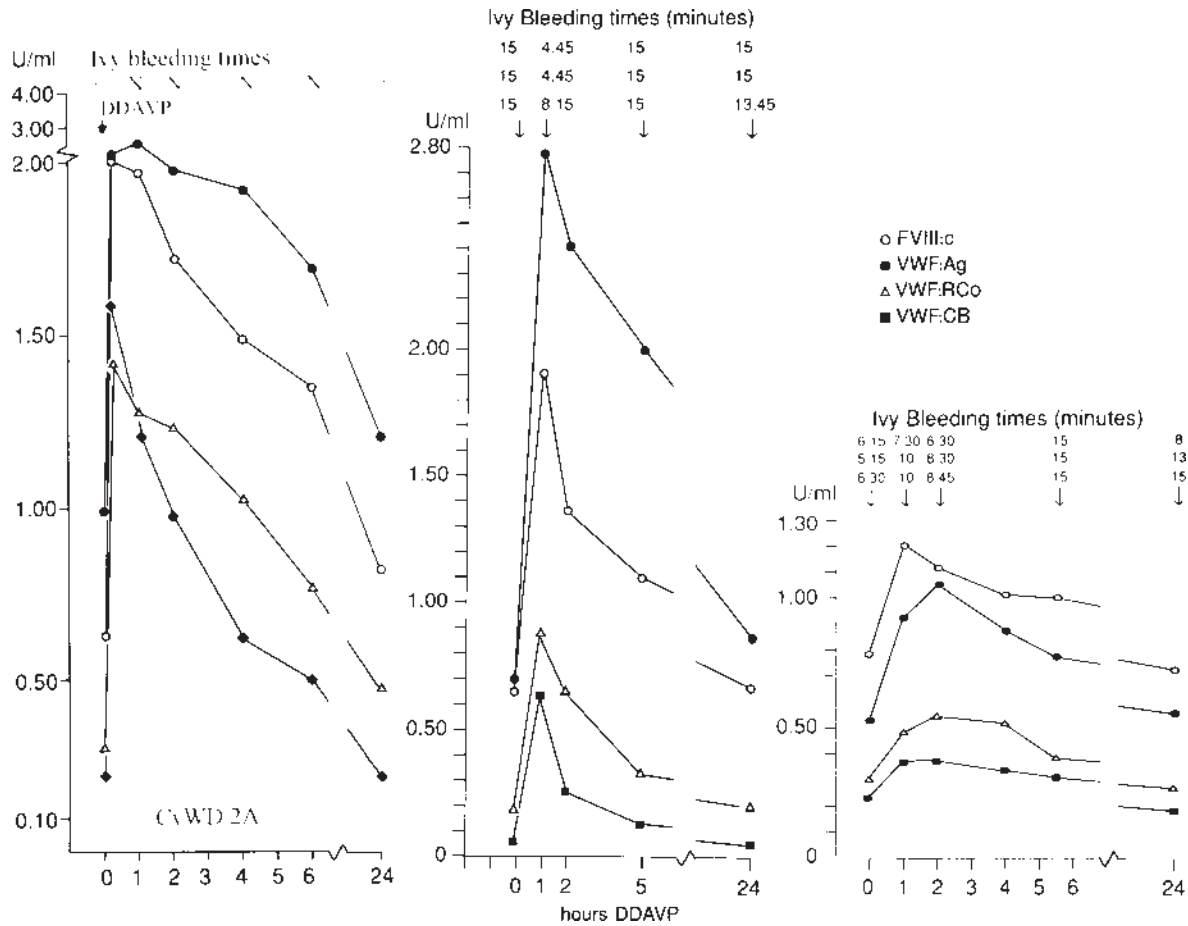


Figure 6 Responses to desmopressin acetate (DDAVP) in a case with mild (left) and two cases with pronounced (middle and right) type 2A von Willebrand disease (vWD) showing normal responses for factor VIII coagulant activity (FVIIIc) and vWF:antigen (Ag) in all, but the responses of vWF:ristocetin cofactor (RCo) and vWF: collagen-binding factor (CB) are transient in mild 2A vWD (left) and minor (middle) or poor (right) in pronounced 2A vWD.

In the studies of Hilbert et al⁶⁰ and Nishikubo et al,⁶¹ the R1374H and R1374C genotype was classified as variant type 2 vWD featured by low vWF:RCo levels, a discrepant vWF:RCo/vWF:Ag ratio, decreased or absent RIPA, and a moderate decrease of high molecular weight vWF multimers together with a significant increase of the lower vWF multimers due to increased proteolysis of the vWF. The laboratory phenotypes of R1374H and R1374C do not belong to type 2A vWD because their plasma vWF displayed intermediate multimers and satellite bands with normal intensity. The pattern of proteolytic fragments of plasma vWF obtained in reduced gels was normal in patients with the R1374H or R1374C mutation, whereas proteolytic fragments are markedly increased in type 2A vWD.³⁹ The two mutations R1374H and R1374C qualitatively modify the proteolysis of vWF. In addition to the physiological fragmentation of the subunit, the mutated vWF shows the presence of an additional 209-kd species not observed in normal types 1, 2A, 2B, and 2N vWF.⁶¹ The relative loss of large multimers with a normal banding

pattern of each multimer clearly differentiates the vWD patients with the R1374H or R1374C mutation from vWD subtypes 2A and 2B with absence of large vWF multimers and pronounced triplet structure of bands, from vWD subtypes 2C, 2E, and 2D where satellite bands are absent or reduced in intensity, and from subtype 2D where satellite bands migrate in an abnormal position.³⁹ Consequently, patients with the R1374H and R1374C mutations are difficult to classify as either type 2M or any other type 2, and therefore are best categorized as type 2U vWD.⁶⁰ The laboratory phenotype of vWD 2U is characterized by decreased values for FVIII:C (mean 32 U/dL), low levels for vWF:Ag (mean 21 U/dL), very low or undetectable levels for vWF:RCo, and the RIPA was uniformly decreased or absent in all cases.^{30,58-62} The response to DDAVP for the patients with mutations R1374H or R1374C has been analyzed occasionally and appeared to be poor for vWF:RCo and somewhat better for vWF:Ag and FVIII:C.⁶³

Since 1998, 23 patients from eight unrelated families with the genetic defect R1315C have been

Table 7 Laboratory Phenotypes and Genetic Defects in the D2 Domain in Six Cases with Autosomal Recessive Type 2C vWD

Author	Age (yr)	Gender	BT (min)	FVIII:C (%)	vWF:Ag	vWF:RCo	vWF:CB (%)	RIPA	MM Pattern
Ruggeri et al ⁶³	39	M	> 30	0.67	0.50	0.10	nt	↓↓	2C
Mutation	Ins405AsnPro (F405insNP)(Holmberg et al ⁵⁹)								
Mazurier et al ⁶⁵	19	F	> 20	0.24	0.16	< 0.03	nt	Zero	2C
Mutation	625insGly/26pdelCT-stop (A625InsG/null) (Gaucher ⁶²)								
Battle et al ⁶⁷	—	M	> 20	0.09	0.13	< 0.01	nt	Zero	2C
	—	F	> 20	0.20	0.15	< 0.01	nt	Zero	2C
Schneppenheim et al ⁶⁹	Child	F	10	> 1.00	1.59	0.25	0.29	nt	2C
Mutation	homozygous G550R								
Gaucher et al ⁶⁶	64	F	> 30	0.20	0.10	< 0.10	nt	nt	2C
Mutation	homozygous Cys623TRP (C623W)								
Mannucci et al ⁷⁰	10	F	> 30	0.85	1.25	0.14	nt	Zero	2C?
Mutation	Unknown								

vWD, von Willebrand disease; BT, bleeding time; FVIII, factor VIII; C, coagulant; vWF, von Willebrand factor; Ag, antigen; RCo, ristocetin cofactor; CB, collagen-binding factor; RIPA, ristocetin-induced platelet aggregation; MM, missense mutation; N, normal; nt, not tested; ↓, decreased.

described in the literature^{30,58,62} and reviewed in great detail.⁵⁶ The laboratory phenotype of the R1315C mutation is characterized by subnormal values for FVIII:C (mean 41 U/dL), low levels for vWF:Ag (mean 20 U/dL), very low or undetectable levels for vWF:RCo of less than 0.10, a decreased vWF:RCo/vWF:Ag ratio between and the RIPA was uniformly decreased or absent in all cases. The R1315C mutation has been labeled as type 2M in two cases by Nitu-Whalley et al.³⁰ The R1315C mutation in 11 affected members of one family has been described by Lethagen et al⁵⁷ as severe type 1 vWD with a poor response to DDAVP and as type 2 variant or 2U by Ribba et al.⁶²

During the 51st Annual Meeting of the ISTH (Sydney, Australia, August 2005), Budde and Schneppenheim⁴⁰ presented a state-of-the-art poster (P2057) entitled "Multimeric pattern in patients diagnosed with type 1 von Willebrand disease in the European study, Molecular and Clinical Markers for the Diagnosis and Management of Type 1 vWD (MCMDM-1vWD)." About half of the patients diagnosed as type 1 have type 2 vWD. So-called type 1 vWD patients are never re-diagnosed as type 2A, 2B, 2C, or 2D, but as type 2E, 2M, or 2A-like vWD. None of the heterozygous mutations of the D1, D2, and D' domains show an abnormal vWF multimeric pattern. The multimerization defects caused by mutations in the D1 or D2 domain are inherited recessively and correlate with a particular phenotype, namely, 2C (see Table 5; Fig. 5). Mutations in the D3 domain (C1130R, C1130G, W1144G, Y1146C) show a type 2E pattern with relative loss of large vWF multimers and reduced triplet structure and proteolysis (Table 5; Fig. 5). Mutations in the A1 to A3 domain (R1315C, R1374C, G1415D) show a relative loss of large vWF multimers, no increase of triplet structure, and a defect of the ristocetin cofactor activity

and RIPA, suggesting a type 2A-like or 2U vWD (Table 5; Fig. 5). Mutations in the D4-CK domain (G2441C, R2464C, R2464X, C2469P) show a type 2M multimeric pattern with the presence of high vWF multimers, no triplet structure, and a smeary pattern. These new observations indicate that the reported cases of type 2M with a mutation in the A1-A3 domain are uniformly associated with reduced vWF:RCo and decreased or absent RIPA, and therefore are to be relabeled as type 2A-like or 2U, and that type 2M is now much better defined as a distinct laboratory phenotype of vWD (Table 5; Fig. 5). The results of the MCMDM-1vWD studies surely will show the manifold phenotypic expressions of types 1 and 2 vWDs, which have important implications with regard to classification and responses of DDAVP with subsequent consequences for the prevention and treatment of bleeding complications.

RECESSIVE TYPE 2C vWD

vWD type 2C shows a characteristic multimeric pattern with a lack of high molecular weight multimers, the presence of one single-banded multimer instead of triplets, and there is a pronounced first band that probably includes a dimer and a tetramer (Fig. 5; Table 5).^{39,40} Autosomal recessive vWD type 2C is caused by homozygosity for a missense mutation (or double heterozygosity of a null allele and missense mutation) in the D1 and D2 domains of the vWF propeptide that catalyzes the multimerization in the D3 domain at the N terminus of mature vWF.³⁶ The laboratory phenotypes and the genetic defect of six cases of autosomal recessive vWD type 2C are shown in Table 7.⁶⁴⁻⁶⁹ Autosomal recessive type 2C vWD, which affects both males and females, is characterized by very low levels of FVIII:C and vWF:Ag, unmeasurable

vWF:RCo, absent RIPA, and strongly prolonged BT in 3 kindreds and with prolonged BT, normal values for FVIII:C and vWF:Ag, and low levels for vWF:RCo and vWF:CB in two kindreds (Table 7). The response to DDAVP in these two cases of autosomal recessive type 2C vWD was very poor, with no increase of vWF parameters and no correction of the BT.^{67,68}

Mannucci et al⁷⁰ described a variant of autosomal recessive type 2 vWD characterized by the lack of large multimers and missing triplet structure in high-resolution agarose (2.5%) gels distinct from types 2C and 2D (Table 7).

DOMINANT TYPE 2D vWD

Autosomal dominant type 2D is rare and only two cases have been reported so far. Typical features of type 2D vWD are the lack of high molecular weight multimers and the presence of a characteristic intervening subband between individual oligomers (Fig. 5; Table 3).^{39,40} The first kindred, a 23-year-old woman and her daughter, have a history of easy bruising, epistaxis, prolonged bleeding from minor injuries, menorrhagia, and postpartum hemorrhage since early childhood.⁷¹ The laboratory phenotype of this kindred was characterized by normal values for FVIII:C and vWF:Ag, moderately decreased vWF:RCo, decreased RIPA, and prolonged BT. The second case of type 2D vWD is a 13-year-old girl with epistaxis since early childhood and heavy menorrhagia since menarche.⁷² The laboratory phenotype was characterized by normal levels for FVIII:C, vWF:Ag, vWF:RCo, low vWF:CB, and prolonged BT. The patient was heterozygous for Cys2010Arg, whereas her mother, father, and sister were asymptomatic and displayed the wild-type sequence of the vWF gene, indicating a *de novo* mutation and excluding autosomal recessive inheritance.⁷² No additional deviant sequences were detected in the remaining 51 exons of the vWF gene, thereby excluding double heterozygosity. The same Cys2019Arg was found in a Swedish family with type 2D vWD.⁷³ The inheritance of the dimerization defect at the C-terminal end of the vWF gene (type 2D) is dominant and homozygous mutations may manifest as vWD type 3.⁷³

DOMINANT TYPE 2E vWD

Zimmerman et al³⁸ first described the only kindred with autosomal dominant mild or asymptomatic type IIE vWD, which is characterized by prolonged BT, normal FVIII:C, and moderately decreased vWF parameters with a normal vWF:RCo/vWF:Ag ratio. The large vWF multimers are missing and the pattern of the individual multimers shows only one clearly identifiable band; there is no intervening band, and a marked increase in the smallest oligomer, a hallmark of type

2C, is not present (Fig. 5; Table 5).^{38–40} This phenotypic multimeric structure of type 2E vWD is caused by reduced proteolytic cleavage.

Schneppenheim et al,³⁹ and Budde and Schneppenheim⁴⁰ have produced preliminary unpublished data that type 2E vWD is associated with a cluster of mutations in the vWF D3 domain that affect mainly cysteine residues that potentially are participating in intermolecular disulfide binding at the N-terminus of the mature vWF subunits as the essential domain of vWF multimerization (Y1146C, C1153Y). The type 2E phenotype is rather common and one third of patients with type 2 vWD have this characteristic.^{39,40} Tjernberg et al²⁷ investigated the effect of C1130F and C1149R mutations in the multimerization area (D3 domain, Fig. 5) in transfection studies of human recombinant vWF. The C1130F and C1149R mutations indeed showed impaired multimerization by the lack of high molecular weight multimers, but also a pronounced secretion defect caused by intracellular retention and degradation of mutant vWF consistent with a quantitative vWD phenotype.^{24–27} The multimeric pattern in a 1:1 ratio cotransfection (mimicking heterozygosity) showed the absence of the highest molecular weight vWF multimers,²⁷ which, according to Eikenboom et al^{24–26} (who used low-resolution agarose gel), corresponds to heterozygous type 1 vWD, and according to Budde et al^{39,40} (who used his sensitive method of multimeric analysis), very likely will represent type 2E vWD. Balloïd et al⁷⁴ and Gaucher et al⁷⁵ identified the Y1107C mutation in the D3 domain in the described type 2-Bern, which showed a multimeric pattern consistent with autosomal dominant type 2E vWD. The variant described by Castaman et al⁷⁶ as autosomal dominant type 1 showed a typical type 2 multimeric pattern with the absence of large multimers and an aberrant triplet structure consistent with type 2E. The subtype IIC Miami can best be regarded as a variant type 2E, differing from type 2E vWD by the elevated vWF:Ag level.⁷⁷ The laboratory phenotype of vWD Miami features prolonged BT, high values for FVIII:C and vWF:Ag, subnormal values for vWF:RCo, and a normal RIPA. The penetrance of bleeding manifestations in the vWD Miami family was variable.

RECESSIVE TYPE 2E-LIKE vWD

In the 1980s, three rare case reports of autosomal recessive types IIG, IIH, and IIF were published, which show a type 2E-like laboratory phenotype.^{78–80} In addition to a decrease or the absence of high molecular weight multimers, the multimeric patterns of subtypes IIE, IIG, IIF, and IIH show an aberrant triplet structure with a lack of outer bands but with pronounced inner banding in high-resolution gels. According to Schneppenheim et al³⁹ and Budde and

Table 8 FVIII:C and vWF Levels Contained in Cryoprecipitates and FVIII/vWF Concentrates; In Vitro Study 1994

FVIII-vWF Concentrate	FVIII:C (U/mL)	vWF:Ag/FVIII:C (U)	vWF:RCo/FVIII:C (U)	vWF:CB/FVIII:C (U)	Ratio vWF:RCo/Ag	Ratio vWF:CB/Ag
Wet cryoprecipitate						
Blood bank 1	9.70 ± 1.72	4.58 ± 1.17	3.87 ± 0.56	3.99 ± 0.47	0.84	0.87
Blood bank 2	2.34 ± 0.15	2.76 ± 0.21	2.69 ± 0.41	2.61 ± 0.34	0.97	0.94
Blood bank 3	8.62 ± 0.55	5.00 ± 0.97	4.19 ± 0.54	4.28 ± 0.50	0.84	0.85
Lyophilized cryoprecipitate	3.65 ± 0.21	4.22 ± 0.74	2.07 ± 0.36	2.40 ± 0.57	0.49	0.58
FVIII/vWF concentrates						
Biotransfusion	23.15 ± 1.59	3.82 ± 0.44	4.30 ± 0.30	3.81 ± 1.13	1.13	0.99
Nordic	47.91 ± 2.75	7.47 ± 1.97	3.59 ± 0.33	4.76 ± 0.96	0.48	0.63
Alpha VIII 250	55.44 ± 4.53	7.11 ± 1.13	3.79 ± 0.20	3.51 ± 0.43	0.53	0.49
Hemate-P	27.33 ± 4.08	2.74 ± 0.14	2.31 ± 0.37	2.66 ± 0.22	0.84	0.97
Monoclote	94.63 ± 3.08	0.40 ± 0.02	< 0.05	< 0.10		

FVIII, factor VIII; vWF, von Willebrand factor; C, Coagulant; Ag, antigen; RCo, ristocetin cofactor; CB, collagen-binding factor.

Schneppenheim,⁴⁰ the phenotype IIG/IIF/IIH may readily be taken together as autosomal recessive 2E different from type 2A (Fig. 5, Table 5). vWD type 2E appears to be less well defined, is usually autosomal dominant, may be recessive, and accounts for about one third of patients with 2A in a large cohort of vWD patients diagnosed in the coagulation laboratory in Hamburg, Germany.^{39,40}

RECESSIVE TYPE 2N vWD

Type 2N vWD encompasses all patients with FVIII:C deficiency caused by a markedly decreased affinity of vWF for FVIII:C.^{81,82} The laboratory phenotype of classical type 2N vWD is characterized typically by reduced FVIII:C levels despite normal or near-normal vWF:Ag, vWF:RCo, and vWF:CB levels; a normal vWF multimeric pattern; and normal vWF-dependent platelet functions including RIPA and bleeding time. The FVIII:C levels range from 1 to 40 U/dL and depend on the severity of the FVIII:vWFCB defect. Although some cases of have severe FVIII:C deficiency of 1 to 2 U/dL, the majority of 2N vWD patients have FVIII:C levels above 5 U/dL. Consequently, type 2N vWD may be misclassified as mild hemophilia. Such patients generally are considered as sporadic cases of hemophilia A or hemophilia A carriers, and the only definitive way to distinguish between mild hemophilia and 2N is to measure FVIII:vWFCB.⁸¹⁻⁸⁴ The bleeding manifestations of type 2N vWD primarily depend on the degree of decreased FVIII:C levels, and therefore mimic those of patients with mild hemophilia A. The most frequent bleeding manifestations of type 2N vWD patients include recurrent epistaxis; bruising, hematomas after trauma; excessive bleeding after minor injuries; bleeding after tonsillectomy and tooth extraction; occasionally hemarthrosis and muscle bleedings; and for affected women, menorrhagia, menorrhagia after the first menstruation, and bleeding after delivery.⁸⁰

The FVIII binding site has been located on the terminal part of the mature vWF subunit and corresponds to the D' domain and to the N-terminal part of the D3 domain of the pre-pro vWF molecule synthesized by the vWF gene (Fig. 1).^{76,77} The vWF gene defects in type 2N vWD are missense mutations located in exons 18 to 27. Most of the type 2N missense mutations are located in exons 18 to 20 of the FVIII binding domain N-terminal region of the mature vWF, which include R782W, G785E, E787K, C788R, C788Y, T791M, Y795C, M800V, R816W, R816Q, H817Q, R854Q, R854W, C858F, and D879N.^{81,82} A few missense mutations of type 2N vWD (1053, 1061, and 1225) are located outside the FVIII binding domain, suggesting that such mutations affect the FVIII binding site, probably by changing its conformation.⁸²

The inheritance of vWD type 2N is autosomal recessive. Type 2N vWD patients are homozygous or double homozygous for the 2N missense mutation or double heterozygous for one 2N and one null vWF allele.⁸⁰⁻⁸³ In type 2N vWD, the FVIII:vWF binding is either nil or dramatically reduced.⁸¹⁻⁸⁴

DDAVP IN TYPE 2 vWD

The responses to DDAVP of FVIII:C and vWF parameters in patients with pseudo-vWD (Fig. 2), type 1 vWD (Fig. 3), and type 2M vWD (Fig. 4) have been described in previous sections of this article. Mild type 2A vWD has a transiently complete response to DDAVP that may be good enough for the treatment and prophylaxis of minor bleedings (Table 6; Fig. 6).^{22,46,47} Most patients with type 2A vWD show only a transient minor or a poor response to DDAVP, with no correction of the BT despite some increase of vWF:RCo (Fig. 6), and therefore are candidates for FVIII/vWF concentrate substitution for the treatment and prophylaxis of bleeding symptoms.^{22,46,47} Federici et al⁸⁵ demonstrated that in

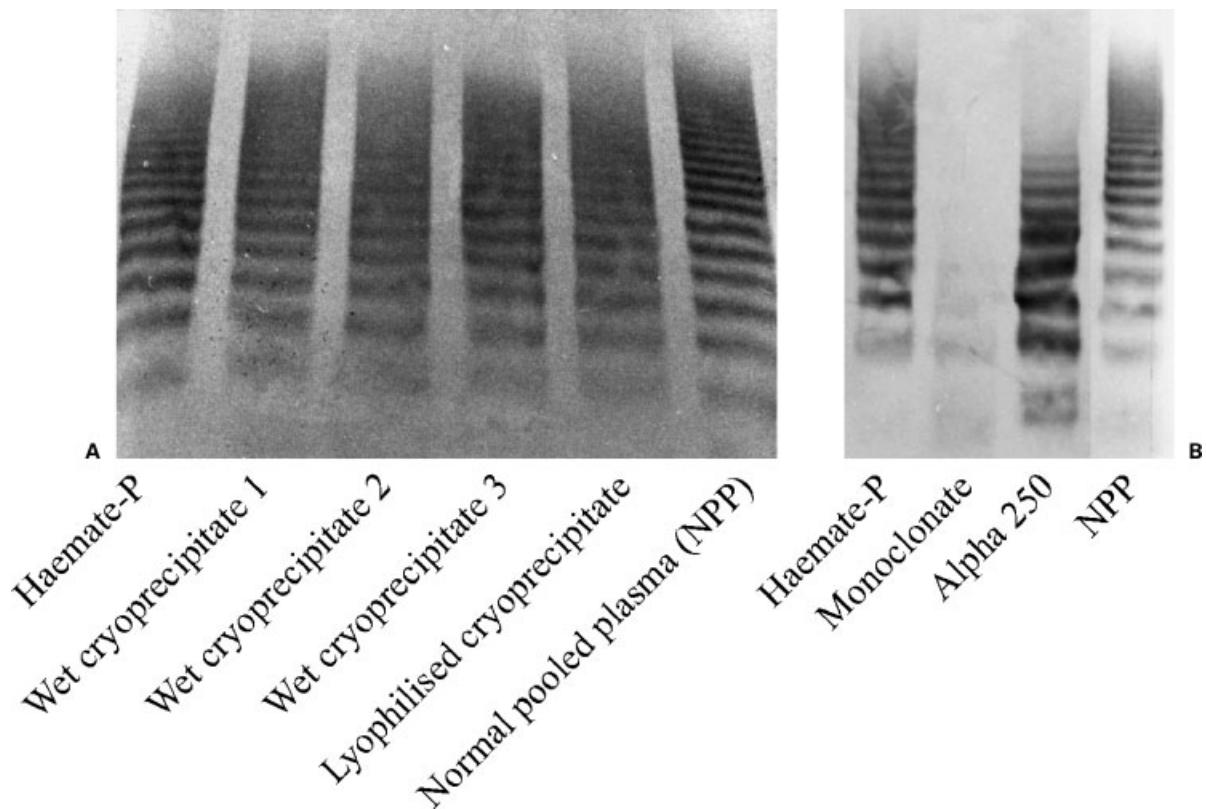


Figure 7 von Willebrand factor (vWF) multimeric patterns of factor VIII (FVIII)/vWF concentrates show the presence of high vWF multimers in cryoprecipitate, Haemate-P, Biotransfusion, and normal plasma, the absence of high vWF multimers in Alpha 250, the absence of vWF in Monoclate, and the presence of vWF degradation products in Alpha and Nordic.

type 2A vWD patients with the mutations S1506L and V1665E causing group 1 defects, the response to DDAVP is poor, with no reappearance of the large multimers and with persistence of a strongly prolonged BT and little increase of vWF:RCo. In contrast, in type 2A vWD patients with the mutations R1597W and G1629R causing group 2 defects, DDAVP induced a transient increase of large multimers associated with a transient (1 or 2 hours) correction of BT and transient correction of vWF:RCo to low normal.

The response to DDAVP in type 2B is normal for FVIII:C and vWF:Ag, transiently good and reaching normal values for vWF:RCo, and subnormal for vWF:CB without correction of BT and no reappearance of large vWF multimers. Therefore type 2B patients are candidates for FVIII/vWF concentrate substitution for the prophylaxis and treatment of bleeding episodes.²²

Recessive type 1 and recessive type 2C vWD are characterized by variable FVIII:C levels, very low vWF:RCo, decreased or absent RIPA, and a very poor response to DDAVP,^{10,67,68} and therefore are candidates for FVIII/vWF concentrates.

Federici et al⁸⁵ evaluated the biological responses in 20 patients with type 2M vWD. Gene mutations were

not known in six and known in 14 of 20 patients: R1315C in three, R1374C in nine, and R1384H in two patients. These three mutations are reported in the literature to be linked with type 2M or 2U vWD.^{48–58} The response to DDAVP was fairly good for FVIII:C, not evaluated for vWF:Ag and vWF:CB, and poor for vWF:RCo in 18 of the 20 so-called type 2M/U vWD patients. The cohort of 20 patients with type 2M vWD had borderline to slightly prolonged BT in seven, prolonged BT in 10, and strongly prolonged (≥ 30 minutes) in three patients. The prolonged BTs were normal at 2 hours after DDAVP in 13 of 20 patients with type 2M vWD.⁸⁵

The response to DDAVP in recessive type 2N vWD of the vWF:Ag, vWF:RCo, and vWF:CB are normal,⁸⁴ but the response of FVIII:C to DDAVP depends on the severity of the FVIII:vWF binding defect. The response to DDAVP of FVIII:C is fairly good in type 2N patients homozygous for the R816W mutation,^{22,84} but the response to DDAVP is poor in type 2N homozygous for the R854Q mutation, and therefore are candidates for FVIII/vWF concentrates.

In conclusion, the responses to DDAVP of FVIII:C and vWF parameters in types 2M, Vincenza, 2E, and mild 2A, 2U, and 2N vWD are transient and

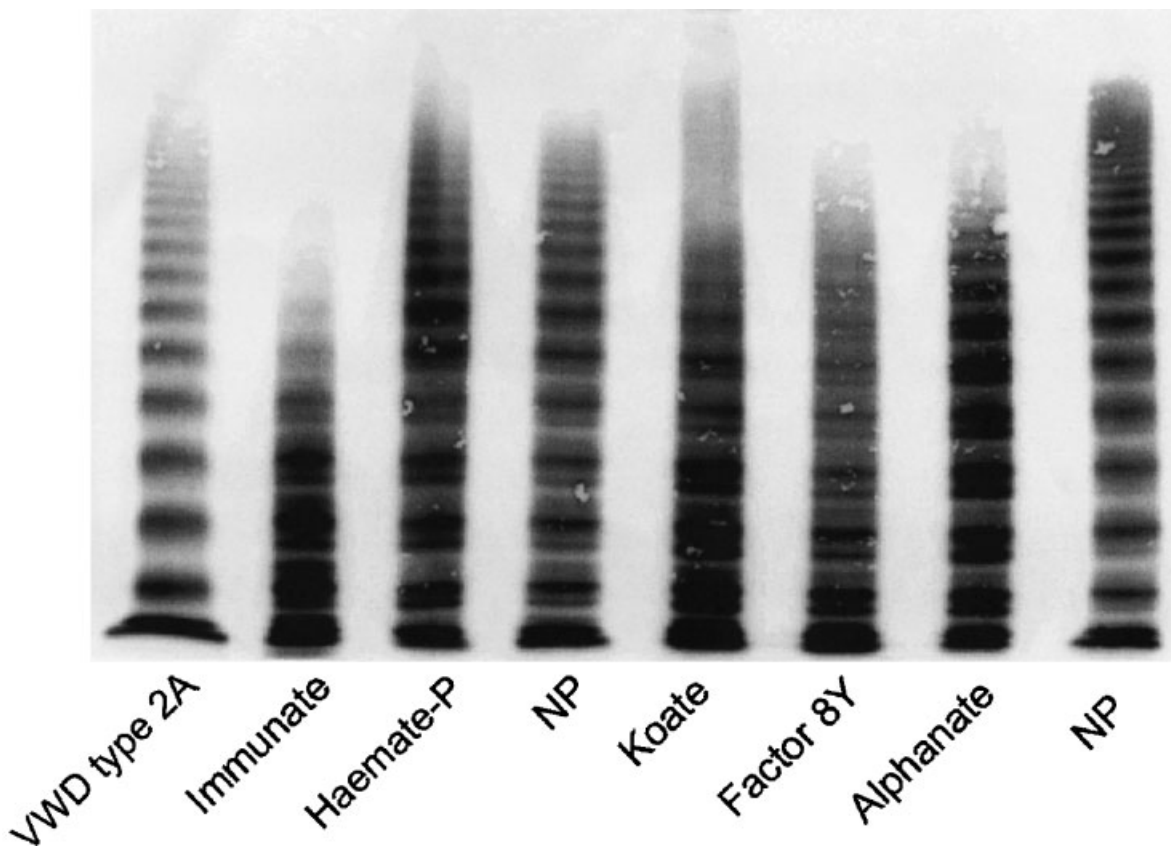


Figure 8 von Willebrand factor (vWF) multimeric patterns of currently available factor VIII (FVIII)/vWF concentrates show how the presence of high vWF multimers in Haemate-P and normal plasma, the absence of high vWF multimers in Koate, Factor 8Y, Alphanate and type 2A vWD, and the absence of high and some of the intermediate vWF multimers in Immunate.

good for a few or some more hours to arrest mucocutaneous bleeding episodes or to prevent bleeding during minor surgery or trauma, but not good enough to treat major bleedings or to prevent bleeding during major surgery or trauma. The responses to DDAVP of FVIII:C and/or vWF parameters in types 2B, 2C, and pronounced cases of 2A and 2U vWD are rather poor to poor, indicating the need to use FVIII/vWF concentrate to treat significant mucocutaneous bleeding episodes or to prevent bleeding during minor and major surgery or trauma. No data on type 2D vWD are available.

FVIII/vWF CONCENTRATES IN vWD TYPES 1, 2, AND 3 WITH A POOR RESPONSE TO DDAVP

vWD patients with recessive type 3, type 1, type 2C, and severe type 2N, and dominant type 2A, 2B, and 2U that respond poorly or not at all to DDAVP are candidates for FVIII/vWF concentrate for the treatment of bleeding episodes and prophylaxis of bleeding during surgery or after minor or major trauma. Cryoprecipitate was the keystone for the treatment of vWD patients from the 1960s until the late 1980s, when the virus-inactivated FVIII/vWF concentrates appeared on the market.^{86,87}

Table 8 shows the content of FVIII:C, vWF:Ag, vWF:RC₀, and vWF:CB (values of three separate assays) in cryoprecipitates and FVIII/vWF concentrates after reconstitution in the amount of fluid recommended by the manufacturer. For wet cryoprecipitates produced in the 1980s and early 1990s by local blood banks in The Netherlands, the concentrations of FVIII:C were lower than those of vWF:Ag (Table 8). The values for vWF:Ag, vWF:RC₀, and vWF:CB were similar, with ratios of vWF:RC₀/vWF:Ag and vWF:CB/vWF:Ag ranging from 0.84 to 0.97 for the three wet cryoprecipitates and 0.49 and 0.58 for the lyophilized cryoprecipitate (Table 8). The multimeric patterns of vWF in the cryoprecipitates compared with a normal plasma run showed a near-normal vWF multimeric pattern, with the presence of high vWF multimers and the absence of vWF degradation products (Fig. 7A). The multimeric patterns of Haemate-P (ZLB-Behring, Marburg, Germany) and normal plasma are quite similar, with the presence of the high-vWF multimers (Fig. 7B).

In 1994, we evaluated four FVIII/vWF concentrates (Biotransfusion, Nordic, Alpha VIII 250 and Hemate-P; Table 8; Fig. 7B). In Biotransfusion and Hemate-P, the multimeric pattern shows equal densities

Table 9 Comparative Analysis of the Calculated In Vivo Recoveries of FVIII:C and vWF:RCo and Their Biological Half-Life Times after a Loading Dose of FVIII/vWF Concentrate for Bleeding Prophylaxis in vWD Patients

Parameter	Authors				
	Auerswald et al ⁹³	Van Elst et al ⁹²	Michiels et al ⁹⁵	Mannucci et al ⁹⁴	
FVIII/vWF concentrate	Immunate	Immunate	Hemate-P	Alphanate SD versus SDHT	
Number of patients	5	7	5	13	
vWD type	Type 2A/B	2A/B	2A/B	Type 3	
Loading dose given in units FVIII/kg body weight	54	40	60		
	50–86	46–63	25–50		
Ratio vWF:RCo to FVIII:C	nt	0.5	2.2	0.625	0.625
Ratio FVIII:C to vWF:RCo	nt	2	0.45	1.6	1.6
Recovery per unit FVIII:C Time after infusion	1 h	0.5 h	1 h	15 min*	
FVIII:C	2.2%	2.1%	3.2%	2.1%	2.9%
	1.6–2.9	1.6–3.8	1.8–4.4	—	—
vWF:RCo	1.3%	nt	3.9%	1.3%	1.8%
	0.6–2.3		3.1–4.6	—	—
vWF:CB (type 1 collagen)	nt	0.65%	2.8%	nt	nt
		0.4–1.0	2.4–3.0		
Recovery per unit vWF:RCo					
FVIII:C	4.4%	4.2%	1.3%	3.4%	4.6%
vWF:RCo	nt	nt	1.7%	2.5%*	2.9%*
vWF:CB	nt	0.35	1.25%	nt	nt
Biological half-life times	Normal	14 h	> 12 h	6.5	7.1 h
$T_{1/2}$ vWF:RCo					

FVIII, factor VIII; C, coagulant; vWF, von Willebrand factor; RCo, ristocetin cofactor; CB, collagen-binding factor SD, solvent detergent; SDHT, solvent detergent, heat treated.

of the low-, intermediate-, and high-vWF multimers, and absence of vWF degradation products (Fig. 7B), which is consistent with normal ratios of vWF:RCo/vWF:Ag and vWF:CB/vWF:Ag ranging from 0.84 to 1.13 (Table 8). In Nordic and Alpha VIII 250, there was a relative excess of vWF:Ag over vWF:RCo and vWF:CB, but decreased ratios of vWF:RCo/vWF:Ag and vWF:CB/vWF:Ag were observed, ranging from 0.48 to 0.63 (Table 8). The multimeric pattern of the vWF in the concentrates Nordic and Alpha VIII 250 (Fig. 7B) showed a relative decrease of the high-vWF multimers together with an abnormal pattern with much more pronounced densities of the low vWF multimers and the presence of vWF degradation band, which is consistent with the lower vWF:RCo/vWF:Ag and vWF:CB/vWF:Ag ratios (Table 8).

The multimeric structure of vWF of the currently available FVIII/vWF concentrates Immunate (Baxter-Immuno, Vienna, Austria), Koate and Factor 8Y (Bio Products Laboratory, Elstree, United Kingdom), and Alphanate (Alpha Therapeutic Corporation, Los Angeles, CA) is abnormal with the exception of Haemate-P are shown in Fig. 8. The multimeric pattern of vWF in the FVIII/vWF concentrate Haemate-P

compared with a normal plasma run shows a near-normal vWF multimeric pattern with equal densities of the low-, intermediate-, and high-vWF multimers (Fig. 8). The multimeric structure of vWF of Immunate shows the absence of the high- and some of the intermediate-vWF multimers, whereas the other currently available FVIII/vWF concentrates Koate, Factor 8Y, and Alphanate show the absence of the high-vWF multimers (Fig. 8).

Prospective comparative pharmacokinetic studies of replacement therapy with various FVIII/vWF concentrates have been conducted for type 3 vWD but not for type 2 vWD patients. FVIII/vWF replacement therapy has been largely empirical, and not tailored to different types of vWD patients, and is not based on FVIII:C for dosing recommendations.^{88–91} The recommendations to treat vWD type 2 patients with FVIII/vWF concentrates are derived from pharmacokinetic studies in type 3 vWD patients. As shown in Table 9, we compared the pharmacokinetic and hemostatic effect of 2 FVIII/von Willebrand factor concentrates (Haemate-P and Immunate) for the treatment of patients with type 2 vWD in four recent studies (Table 9).^{92–95} The calculated in vivo recoveries of

Table 10 Recommendations for the Treatment of Bleeding Episodes and Prophylaxis of Bleeding During Surgery or Trauma in Patients with Recessive Type 3 and 1, and Type 2, Who Respond Poorly to DDAVP: FVIII:C Dosing Versus vWF:RCo Dosing

Italian Recommendations Using FVIII:C Dosing ⁹⁶⁻⁹⁸			
Type 3 vWD Type 2 vWD Not Responsive to DDAVP	Initial Dose FVIII:C (U/kg)	Subsequent Infusion	Objective
Major surgery or trauma	50	Once a day or every other day	Adjust to maintain FVIII:C > 0.50 U/mL until wound healing is reached for at least 7-10 d
Minor surgery or trauma	40	Once a day or every other day	Maintain FVIII:C > 0.30 U/mL for at least 5-7 d
Delivery and puerperium	40	Once a day	Maintain FVIII:C > 50 U/mL for 3-4 d
Dental extraction	30	Single dose	Maintain FVIII:C > 0.30 U/mL for > 12 h
Spontaneous or posttraumatic bleeding	30	Single or daily	Depending on severity FVIII:C > 50 U/mL for 1-3 d
Proposed Recommendations Using vWF:RCo Dosing ⁹⁵			
Type 2A, 2B, 2C, 2U Recessive Type 1 Severe 2N vWD	Initial Dose vWF:RCo	Subsequent Infusions vWF:RCo Dosing	Objective
Major surgery or trauma severe bleeding	60-80	40 U/kg every 12 h for a few days and 30 U/kg for another 2-4 d no tranexamic acid	Adjust to maintain vWF:RCo > 0.60 U/mL until wound healing is normal for 4-7 d
Minor surgery or trauma	40-60	40 U/kg once on day 1, add tranexamic acid	Normal vWF:RCo > 0.50 for 12 h and adequate vWF:RCo for 2 d
Dental extraction	40-60	Single dose, tranexamic acid	Adequate vWF:RCo > 12 h
Mucocutaneous bleeding	40-60	Single dose, tranexamic acid	Adequate vWF:RCo > 12 h
Delivery, puerperium	60	30-40 U/kg daily for 3-4 d	Adequate vWF:RCo > 0.50 U/mL for 4 d
Type 3 vWD	Initial Dose vWF:RCo	Subsequent Infusion vWF:RCo Dosing	Objective
Major surgery or trauma	80-100	40-60 U/kg every 12 h for 1-2 d and 30-40 U/kg/d for another 3-6 d	Adjust to maintain FVIII:C and vWF:RCo > 0.60 U/mL until wound healing is reached (7-10 d)
Minor surgery or trauma musculoskeleton bleeding delivery, puerperium	60	40-60 U/kg once a day for a few days	Maintain FVIII:C and vWF:RCo > 0.50 U/mL for a 3-4 d
Dental extraction	60	40 U/kg once on day 1 for 2 d	Maintain FVIII:C and vWF:RCo > 0.30
Prophylaxis joint bleeds	60	2 or 3 times a week	Maintain FVIII:C > 0.02%

DDAVP, desmopressin acetate; FVIII, factor VIII; vWF, von Willebrand factor; C, coagulant; RCo, ristocetin cofactor; vWD, von Willebrand disease.

Immunate per transfused unit of FVIII:C were 2.2% for FVIII:C and 1.3% vWF:RCo in five type 2 vWD patients in the study of Auerswald et al,⁹³ and 2.1% for FVIII:C and 0.65% for vWF:CB in seven type 2 vWD patients in the study of Ver Elst.⁹² Mean in vivo

recoveries for Haemate-P per transfused unit of FVIII:C/kg body weight were 3.2% for FVIII:C, 3.9% for vWF:RCo, and 2.8% for vWF:CB, indicating a clear superiority of Haemate-P over Immunate.⁹⁵ Dosing of Immunate and Haemate-P with units of

vWF:RCo/kg to reach an equal 1.7% in vivo increase of vWF:RCo will induce an increase in FVIII:C of more than 4.2% for Immunate and only 1.3% for Haemate-P (Table 9). These in vivo data are directly related to their in vitro characteristics of the absence of high- and intermediate-vWF multimers in Immunate and the presence of the large-vWF multimers in Haemate-P, and with the ratio of vWF:RCo to FVIII:C of 0.5 in Immunate^{92,93} and 2.2 in Hemate-P.⁹⁵

The comparative analysis of data in Table 9 demonstrate that treatment of vWD patients with Alphanate⁹⁴ or Immunate^{92,93} using FVIII:C dosing will lead to undertreatment with regard to vWF:RCo, and that treatment with Alphanate or Immunate using vWF:RCo dosing in units per kilogram will result in overtreatment with regard to FVIII:C. When comparing the shortened half-life times for vWF:RCo of 6.5 to 7.1 hours after transfusion of Alphanate⁹⁴ versus approximately 12 hours for Haemate-P,⁹⁵ it becomes clear to clinicians that sufficient hemostatic levels of vWF:RCo persist with Haemate-P for a much longer time. For repeated doses (e.g., major surgery in vWD), clearance becomes even more important, leading to a significantly lower amount of overall Haemate-P required when compared with Alphanate.

The Italian recommendations in Table 10⁹⁶⁻⁹⁸ for the treatment of severe types 1, 2, and 3 vWD that are not responsive to DDAVP using FVIII:C dosing is problematic and has different implications for the various FVIII/vWF concentrates; reaching the recommended adequate FVIII:C levels, with regard to vWF:RCo levels, will result in either overtreatment when using Haemate-P (with a vWF:RCo/FVIII:C ratio of 2.1 to 2.5) or to undertreatment when using Alphanate or Immunate (with a vWF:RCo/FVIII:C ratio of 0.625 and 0.5, respectively). Conversely, given that the average FVIII:C/vWF:RCo ratio is 1.6 for Alphanate⁹⁴ and 2 for Immunate,^{92,93} treatment of vWD patients with the recommended dose of 60 U of vWF:RCo/kg to reach normal values for vWF:RCo of approximately 1.0 to 1.2 U/mL, will result in much higher and very high levels of FVIII:C above 2.0 U/mL up to 3.0 to 4.0 U/mL for both Alphanate⁹⁴ and Immunate.^{92,93,96} Such high FVIII:C levels for more than 1 week after treatment with FVIII/vWF concentrate for major surgery in vWD patients is a plausible explanation for the increased risk of postoperative venous thrombosis. Because the average FVIII:C/vWF:RCo ratio is 0.40 to 0.45 for Hemate-P,⁹⁵ treatment of vWD patients with the recommended dose of 60 U of vWF:RCo/kg to reach normal values for vWF:RCo of approximately 1.0 to 1.2 U/mL will result in much lower recoveries of FVIII:C during prolonged prophylaxis after major surgery or trauma in type 2 and type 3 vWD patients.⁹⁹⁻¹⁰¹

Given that the functional vWF parameters vWF:RCo and vWF:CB are very low, and the levels of vWF:Ag and FVIII:C are just below or in the normal range in type 2 vWD (Figs. 3, 4, and 6), the Italian recommendation using FVIII:C dosing cannot be applied for these type 2 vWD patients. vWF:RCo dosing should be used in type 2 vWD for proper monitoring of the effect of the various FVIII/vWF concentrates for the treatment of bleeding episodes and bleeding prophylaxis during and after surgery or trauma. Therefore, we propose adjusted recommendations to dose FVIII/vWF concentrates in units of vWF:RCo/kg body weight for the treatment and prophylaxis of bleeding in type 2 vWD with a poor response to DDAVP, and extended it to recessive type 1 and type 3 vWD (Table 10).⁹⁵ The adjusted recommendations are tailored for the severity of bleeding complications, for the type of surgery (minor or major), and for type 2 and type 3 vWD patients (Table 10).⁹⁵ Evaluation of these recommendations in prospective management studies together with labeling of the hemostatic potency of FVIII/vWF concentrates by the content of FVIII:C, vWF:Ag, vWF:RCo, vWF:CB, and vWF multimeric patterns are warranted and are of enormous importance.

Finally, Goudemand et al¹⁰² evaluated the pharmacokinetic response of one loading dose of high-purity vWF concentrate (63 U/kg vWF:RCo and 4 U/kg FVIII:C) in one patient with type 3 vWD. Maximum levels of vWF:RCo and vWF:Ag were observed 1 hour after infusion, whereas maximum levels of FVIII:C were attained from 6 to 12 hours after the first infusion. The biological half-life times ($T_{1/2}$) of FVIII:C, vWF:Ag, and vWF:RCo were 74, 21, and 18 hours, respectively. In subsequent pharmacokinetic studies in type 3 vWD patients infused with purified vWF product (LFB; Lille, France), 10 infusion studies at doses of 50 or 100 U of vWF:RCo/kg body weight revealed that FVIII:C synthesized (was bound to the infused vWF) at 0.6 U/dL/h and decayed with a half-life of 16 hours, that the infused vWF:Ag and vWF:RCo decayed with a half-life of 12 hours.^{103,104} Wilfactin (LFB, Lille, France), a very high purity (VHP) vWF concentrate, has been demonstrated to be effective in type 2 and type 3 vWD patients. A loading dose of 50 to 60 U of vWF:RCo/kg resulted in vWF:RCo levels of > 1.00 U/mL followed by correction of FVIII:C to > 0.50 after 12 hours. Surgery was covered with an infusion, 1 hour preoperatively (mean initial dose 51 to 55 U of vWF:RCo/kg), in type 2 vWD (with FVIII:C > 0.20 to > 0.30 U/mL) for surgery. The mean postinfusion levels were 1.00 U/mL for vWF:RCo and > 0.50 U/mL for FVIII:C. Patients with FVIII:C below 0.20 to 0.30 U/mL received either one infusion of VHP vWF concentrate given 12 to 24 hours preoperatively, or one infusion of FVIII 1 hour preoperatively. The vWF:RCo was increased to > 1.00 U/mL and FVIII:C was > 0.60 in all patients. By maintaining vWF:RCo at

approximately 1.00 U/mL for 1 to 16 days, depending on the procedure, no additional FVIII was required and all patients had adequate hemostasis.¹⁰⁵

REFERENCES

- Goodeve A, Peake I. A standard nomenclature for von Willebrand factor gene mutations and polymorphisms. *Baillieres Clin Haematol* 2001;14:235–240
- Sadler JE. A revised classification of von Willebrand disease. *Baillieres Clin Haematol* 1994;71:520–525
- Sadler JE, Mannucci PM, Berntorp E, et al. Impact, diagnosis and treatment of von Willebrand Disease. *Thromb Haemost* 2000;84:160–174
- Zhang Z, Lindstedt M, Blombäck M, Anvret M. Effects of mutant von Willebrand factor gene in von Willebrand disease. *Hum Genet* 1995;96:388–394
- Schneppenheim R, Krey S, Bergmann F, et al. Genetic heterogeneity of severe von Willebrand disease type III in the German population. *Hum Genet* 1994;94:640–652
- Eikenboom JCJ. Congenital von Willebrand disease type 3: clinical manifestations, pathophysiology and molecular biology. *Baillieres Clin Haematol* 2001;14:365–379
- Lak M, Peyvandi F, Mannucci PM. Clinical manifestations and complications of childbirth and replacement therapy in 385 Iranian patients with type 3 von Willebrand disease. *Br J Haematol* 2000;111:1236–1239
- Eikenboom JCJ, Castaman G, Vos HL, Bertina RM, Rodeghiero FL. Characterisation of genetic defects in recessive type 1 and type 3 von Willebrand disease patients of Italian origin. *Thromb Haemost* 1998;79:709–717
- Allen S, Abuzenadah AM, Hinks J, et al. A novel von Willebrand disease-causing mutation (Arg273Trp) in the von Willebrand factor propeptide that results in defective multimerisation and secretion. *Blood* 2000;96:560–568
- Castaman G, Lattuada A, Mannucci PM, Rodeghiero F. Factor VIII:C increases after desmopressin in a subgroup of patients with autosomal recessive severe von Willebrand disease. *Br J Haematol* 1995;89:147–151
- Castaman G, Eikenboom JCJ, Lattuada A, Manucci PM, Rodeghiero F. Heightened proteolysis of the von Willebrand factor subunit in patients with von Willebrand disease hemizygous or homozygous for the C2364F mutation. *Br J Haematol* 2000;108:188–190
- Castaman G, Novella E, Castiglia E, Eikenboom JCJ, Rodeghiero F. A novel family with recessive von Willebrand disease due to compound heterozygosity for a splice site mutation and a missense mutation in the von Willebrand factor gene. *Thromb Res* 2002;105:135–138
- Schneppenheim R, Budde U, Obser T, et al. Expression and characterization of von Willebrand factor dimerization defects in different types of von Willebrand disease. *Blood* 2001;97:2059–2066
- Eikenboom JCJ, Reitsma PH, Peerlinck KJM, Briet E. Recessive inheritance of von Willebrand's disease. *Lancet* 1993;341:982–986
- Gill JC, Endres-Brooks J, Bauer PJ, Marks WJ, Montgomery JR. The effect of ABO bloodgroup on the diagnosis of von Willebrand disease. *Blood* 1987;69:1691–1695
- Sadler JE. Von Willebrand disease type 1: a diagnosis in search of a disease. *Blood* 2003;101:2089–2093
- Sadler JE. New concepts in von Willebrand Disease. *Annu Rev Med* 2005;56:173–191
- Coughlan TC, Blagg JL, Alulola M, et al. Null alleles are not a common cause of type 1 von Willebrand disease in the British population. *Thromb Haemost* 1999;82:1373–1375
- O'Brien LA, James PD, Hemophilia Clinical Directors Canada, Lillicrap D. Founder von Willebrand factor haplotype associated with type 1 von Willebrand disease. *Blood* 2003;102:549–557
- Bowen D. Type 1 von Willebrand disease: a possible novel mechanism. *Blood Coagul Fibrinolysis* 2004;14(suppl 1):S21–S23
- Bowen D, Collins PW, Lester W, et al. The prevalence of the cysteine 1584 variant of von Willebrand factor is increased in type 1 von Willebrand disease: co-segregation with increased susceptibility to 13 proteolysis but not clinical phenotype. *Br J Haematol* 2005;128:830–836
- Michiels JJ, Van De Velde A, Van Vliet HHDM, Van Der Planken M, Schroyens W, Berneman ZN. Response of von Willebrand factor parameters to desmopressin in patients with type 1 and type 2 congenital von Willebrand disease: diagnostic and therapeutic implications. *Semin Thromb Hemost* 2002;28:111–131
- Eikenboom JCJ, Castaman G, Kamphuisen PW, Rosendaal FR, Bertina RM. The factor VIII/von Willebrand factor ratio discriminates between reduced synthesis and increased clearance of von Willebrand factor. *Thromb Haemost* 2002;87:252
- Eikenboom JCJ, Matsushita T, Reitsma PH, et al. Dominant type 1 von Willebrand disease caused by mutated cysteine residues in the D3 domain of von Willebrand factor. *Blood* 1996;88:2433–2441
- Bodo I, Katsumi A, Tuley EA, Eikenboom JCJ, Dong Z, Sadler JE. Type 1 von Willebrand disease mutation Cys1149Arg causes intracellular retention and degradation of heterodimers: a possible general mechanism for dominant mutations of oligomeric proteins. *Blood* 2001;98:2973–2978
- Castaman G, Eikenboom JCJ, Missiaglia E, Rodeghiero F. Autosomal dominant type 1 von Willebrand disease due to G3639T mutation (C1130) in exon 26 of von Willebrand gene: description of five Italian families and evidence for a founder effect. *Br J Haematol* 2000;108:876–879
- Tjernerberg P, Vos HL, Castaman G, Bertina RM, Eikenboom JCJ. Dimerization and multimerization defects of von Willebrand factor due to mutated cysteine residues. *J Thromb Haemost* 2004;2:257–265
- Casana P, Martínez F, Haya S, Espinos C. Association of the 3467C > T mutation (T1156M) in the von Willebrand's factor gene with dominant type 1 von Willebrand's disease. *Ann Hematol* 2001;80:381–383
- Federici AB, De Groot PhG, Moia M, Ijssedijk MJW, Sixma JJ, Mannucci PM. Type 1 von Willebrand disease, subtype 'platelet low': decreased platelet adhesion can be explained by low synthesis of von Willebrand factor in endothelial cells. *Br J Haematol* 1993;83:88–93
- Nitu-Whalley IC, Ridell A, Lee C, et al. Identification of type 2 von Willebrand disease in previously diagnosed type 1 patients: a reappraisal using phenotypes, genotypes and molecular modelling. *Thromb Haemost* 2000;84:998–1004
- Mannucci PM, Lombardi R, Castaman G, et al. Von Willebrand disease "Vicenza" with larger-than-normal (Supranormal) von Willebrand factor multimers. *Blood* 1988;71:65–70

32. Casonato A, Pontara E, Sartorelo F, et al. Reduced von Willebrand factor survival in type Vicenza von Willebrand disease. *Blood* 2002;99:180–184
33. Cattaneo M, Federici AB, Lecchi A, et al. Evaluation of the PFA-1000 system in the diagnosis and therapeutic monitoring of patients with von Willebrand disease. *Thromb Haemost* 1999;82:35–39
34. Schneppenheim R, Federici A, Budde U, et al. von Willebrand disease type 2M “Vicenza” in Italian and German patients: identification of the first candidate mutation (G3864A; R1205H) in 8 families. *Thromb Haemost* 2000;82:136–140
35. Michiels JJ, Berneman Z, Gadisseur A, et al. Characterization of recessive severe type 1 and 3 von Willebrand disease (VWD), asymptomatic heterozygous carriers versus blood-group O related von Willebrand factor deficiency and dominant type 1 VWD. *Clin Applied Thromb Hemost* 2005; (in press)
36. De Wit TR, Van Mourik JA. Biosynthesis, processing and secretion of von Willebrand factor: biological implications. *Baillieres Clin Haematol* 2001;14:241–255
37. Ruggeri ZM. Structure of von Willebrand factor and its function in platelet adhesion and thrombus formation. *Baillieres Clin Haematol* 2001;14:257–279
38. Zimmerman ThS, Dent JA, Ruggeri ZM, Nannini LH. Subunit composition of plasma von Willebrand factor. *J Clin Invest* 1986;77:947–951
39. Schneppenheim R, Budde U, Ruggeri ZM. A molecular approach to the classification of von Willebrand disease. *Baillieres Clin Haematol* 2001;14:281–298
40. Budde U, Scheneppenheim R. Phenotypic and genotypic diagnosis of von Willebrand disease: a 2004 update. *Semin Hematol* 2005;42:15–28
41. Nichols W, Ginsburg D. von Willebrand disease. *Medicine* 1997;76:1–20
42. Meyer D, Fressinaud E, Gaucher C, et al. Gene defects in 150 unrelated French cases with type 2 von Willebrand disease: from the patient to the gene. *Thromb Haemost* 1997;78:451–456
43. Meyer D, Fressinaud E, Hilbert L, Ribba A-S, Lavergne J-M, Mazurier C. Type 2 von Willebrand disease causing defective von Willebrand factor-dependent platelet function. *Baillieres Clin Haematol* 2001;14:349–364
44. Castaman G, Federici AB, Rodeghiero F, Mannucci PM. von Willebrand’s disease in the year 2003: towards the complete identification of gene defects for correct diagnosis and treatment. *Haematologica* 2003;88:94–108
45. Lyons SE, Bruck ME, Bowie EJW, Ginsburg D. Impaired intracellular transport by a subset of type IIA von Willebrand disease mutations. *J Biol Chem* 1992;267:4424–4430
46. Batlle J, Lopez-Fernandez MF, Campos M, et al. The heterogeneity of type IIA von Willebrand’s disease. *Blood* 1986;68:1207–1212
47. Batlle J, Lassieri J, Villamor AF, et al. Proteolytic processing of von Willebrand factor subunit: heterogeneity in type IIA von Willebrand disease. *Ann Hematol* 1994;68:111–115
48. Howard MA, Salem HH, Thomas KB, et al. Variant von Willebrand’s disease type B—revisited. *Blood* 1982;60:1420–1428
49. Rabinowitz I, Tuley EA, Mancuso DJ, et al. von Willebrand disease type B: a missense mutation selectively abolishes ristocetine-induced von Willebrand binding to platelet glycoprotein Ib. *Proc Natl Acad Sci USA* 1992;89:9846–9849
50. Firkin B, Firkin F, Stott L. von Willebrand’s disease type B: a newly defined bleeding diathesis. *Aust N Z J Med* 1973;3:225–229
51. Mannucci PM, Lombardi R, Bader R, et al. Heterogeneity of type I von Willebrand disease: evidence for a subgroup with abnormal von Willebrand factor. *Blood* 1985;66:796–802
52. Federici AB, Canciani MT, Forza I, Cozzi G. Ristocetin cofactor and collagen binding activities normalized to antigen levels for a rapid diagnosis of type 2 von Willebrand disease. *Thromb Haemost* 2000;84:1127–1128
53. Mancusco DJ, Kroner PA, Christopherson PM, Vokac EA, Gill JC, Montgomery RR. Type 2M:Milwaukee-1 von Willebrand disease with an in-frame deletion in the Cys509–Cys695 loop of the von Willebrand factor A1 domain causes deficient binding of von Willebrand to platelets. *Blood* 1996;88:2559–2568
54. Hillery CA, Mancusco DJ, Sadler JE, et al. Type 2M von Willebrand disease: F606I and I662F mutations in the glycoprotein Ib binding domain selectively impair ristocetin—but not botrocetin-mediated binding of von Willebrand factor to platelets. *Blood* 1998;91:1572–1581
55. Hilbert L, Jenkins PV, Gaucher G, et al. Type 2M vWD resulting from a lysine deletion within a four lysine residue repeat in the A1 loop of von Willebrand factor. *Thromb Haemost* 2000;84:188–194
56. Michiels JJ, Berneman Z, Gadisseur A, et al. Classification and characterization of type 2A, 2B, 2C, 2E, 2D, 2M, 2N and 2U (unclassifiable) von Willebrand diseases. *Clin Appl Thromb Hemost* 2005; (in press)
57. Lethagen S, Frick K, Isaksson C, Kristoffersson A-C, Holmberg L. Revised classification and treatment of von Willebrand disease. *Thromb Haemost* 1998;80:199–200
58. Hoyer LW, Rizza CR, Tuddenham GD, Carta CA, Armitage H, Rotblatt F. von Willebrand factor multimer patterns in von Willebrand’s disease. *Br J Haematol* 1983;55:493–507
59. Castaman G, Eikenboom JCJ, Rodeghiero F, Briet E, Reitsma PH. A novel candidate mutation (Arg⁶¹¹ → His) in type I ‘platelet discordant’ von Willebrand disease with desopressine-induced thrombocytopenia. *Br J Haematol* 1995;89:656–658
60. Hilbert L, Gaucher C, Mazurier C. Identification of two mutations (Arg611Cys and Arg611His) in the A1 loop of von Willebrand factor (VWF) responsible for type 2 von Willebrand disease with decreased platelet-dependent function of VWF. *Blood* 1995;86:1010–1018
61. Nishikubo T, Christophe O, Lavergne J-M, et al. Abnormal proteolytic processing of von Willebrand factor Arg611Cys and Arg611His. *Thromb Haemost* 1997;77:174–182
62. Ribba AN, Hilbert L, Lavergne JM, et al. The Arg552Cys (R552C) mutation within the A1 loop of von Willebrand factor (VWF) induces abnormal folding with loss-of-function resulting in 2A-like phenotype of von Willebrand disease. Study of ten patients and of mutated recombinant VWF. *Blood* 2001;97:952–959
63. Ruggeri ZM, Nilsson IM, Lombardi R, Holmberg L, Zimmerman TS. Aberrant multimeric structure of von Willebrand factor in a new variant of von Willebrand’s disease (type IIC). *J Clin Invest* 1982;70:1124–1127
64. Holmberg L, Karpman D, Isakson C, Kristoffersson AC, Lethagen S, Schneppenheim R. Ins405AsnPro mutation in

- the von Willebrand factor propeptide in recessive type 2A (IIC) von Willebrand's disease. *Thromb Haemost* 1998;79:718-722
65. Mazurier C, Manucci PM, Parquet-Gernez A, Goudemand M, Meyer D. Investigation of a case of subtype IIC von Willebrand disease: characterisation of the variability of this subtype. *Am J Hematol* 1986;22:301-311
 66. Gaucher C, Diéval J, Mazurier C. Characterization of von Willebrand factor gene defects in two unrelated patients with type IIC von Willebrand disease. *Blood* 1994;84:1024-1030
 67. Batlle J, Lopez-Fernandez MF, Lasiera J, et al. von Willebrand disease type IIC with different abnormalities of von Willebrand factor in the same sibship. *Am J Hematol* 1986;21:177-188
 68. Batlle J, Lopez Gernandez MF, Fernandez Villamor A, Lopez Berges C, Zimmerman TS. Multimeric pattern discrepancy between platelet and plasma von Willebrand factor in type IIC von Willebrand disease. *Am J Hematol* 1986;22:87-88
 69. Schneppenheim R, Thomas KB, Krey S, et al. Identification of a candidate missense mutation in a family with von Willebrand disease type IIC. *Hum Genet* 1995;95:681-686
 70. Mannucci PM, Lombardi R, Pareti FI, Solinas S, Mazzucconi MG, Mariani G. A variant of von Willebrand's disease characterized by recessive inheritance and missing triplet structure of von Willebrand factor multimers. *Blood* 1983;62:1000-1005
 71. Kinoshita S, Harrison J, Lazerson J, Abildgaard CF. A new variant of dominant type II von Willebrand's disease with aberrant multimeric pattern of factor VIII-related antigen (type IID). *Blood* 1984;63:1369-1371
 72. Schneppenheim R, Brassard J, Krey S, et al. Defective dimerization of von Willebrand factor subunits due to a Cys → Arg mutation in type IID von Willebrand disease. *Proc Natl Acad Sci USA* 1996;93:3581-3586
 73. Schneppenheim R, Budde U, Obser T, et al. Expression and characterization of von Willebrand factor dimerization defects in different types of von Willebrand disease. *Blood* 2001;97:2059-2066
 74. Baillo P, Gaucher C, Affolter B, Mazurier C, Pflugshaupt R. New variant of type II von Willebrand's disease with structural abnormality of plasma von Willebrand factor in a patient with very mild bleeding history. *Am J Hematol* 1995;49:21-28
 75. Gaucher C, Parquet A, Baillo P, Hanss M, Mazurier C. Mutations in the D3 domain of von Willebrand factor are identified in patients classified as 1 or 2A von Willebrand disease. *Thromb Haemost* 1997;(suppl):388 (abst OC-1583)
 76. Castaman G, Rodeghiero F, Lattuada A, Mannucci PM. A new variant of von Willebrand disease (type II I) with a normal degree of proteolytic cleavage of von Willebrand factor. *Thromb Res* 1992;65:343-351
 77. Ledford M, Rabinowitz I, Sadler JE, Kent JW, Civantos F. New variant of von Willebrand disease type II with markedly increased levels of von Willebrand antigen and dominant mode of inheritance: von Willebrand disease type IIC Miami. *Blood* 1993;82:169-175
 78. Mannucci PM, Lombardi R, Federici AB, Dent JA, Zimmerman TS, Ruggeri ZM. A new variant of type II von Willebrand disease with aberrant multimeric structure of plasma but not platelet von Willebrand factor (type IIF). *Blood* 1986;68:269-274
 79. Gralnick HR, Williams SB, McKeown LP, Maisonneuve P, Jeneau C, Sultan Y. A variant of type II von Willebrand disease with an abnormal triplet structure and discordant effects of protease inhibitors on plasma and platelet von Willebrand factor structure. *Am J Hematol* 1987;24:259-266
 80. Federici AB, Manuccio PM, Lombardi R, et al. Type II H von Willebrand disease: new structural abnormality of plasma and platelet von Willebrand factor in a patients with prolonged bleeding time and borderline levels of ristocetin cofactor activity. *Am J Hematol* 1989;32:287-293
 81. Mazurier C. von Willebrand disease masquerading as haemophilia A. *Thromb Haemost* 1992;67:391-396
 82. Mazurier C, Goudemand J, Hilbert L, Caron C, Fressinaud E, Meyer D. Type 2N von Willebrand disease: clinical manifestations, pathophysiology. Laboratory diagnosis and molecular biology. *Baillieres Clin Haematol* 2001;14:337-347
 83. Schneppenheim R, Budde U, Krey S, et al. Results of a screening for von Willebrand disease type 2N in patients with suspected haemophilia A or von Willebrand disease type 1. *Thromb Haemost* 1996;76:598-602
 84. Mazurier C, Gaucher C, Jorioux S, Goudemand M. Biological effect of desmopressin in eight patients with type 2N ('Normandy') von Willebrand disease. *Br J Haematol* 1994;88:849-854
 85. Federici AB, Mazurier C, Berntorp E, et al. Biologic response to desmopressin in patients with severe type 1 and type 2 von Willebrand disease: results of a multicenter European study. *Blood* 2004;103:2032-2038
 86. Mannucci PM, Moia M, Rebulli P, Altieri D, Monteaguda J, Castillo R. Correction of the bleeding time in treated patients with severe von Willebrand disease is not solely dependent on the normal multimeric structure of plasma von Willebrand factor. *Am J Hematol* 1987;25:55-65
 87. Castillo R, Monteaguda J, Escolar G, Ordinas A, Magallon M, Villar JM. Hemostatic effect of normal platelet transfusion in severe von Willebrand disease patients. *Blood* 1991;77:1901-1905
 88. Mannucci PM. Recommended protocol for the study of ex vivo biological effects of virus-inactivated plasma concentrates with von Willebrand disease. *Thromb Haemost* 1992;68:84-88
 89. Mannucci PM, Tenconi PM, Castaman G, Rodeghiero R. Comparison of four virus-inactivated plasma concentrates for treatment of severe von Willebrand disease: a cross-over randomized trial. *Blood* 1992;79:3130-3137
 90. Lethagen S, Berntorp E, Nilsson IM. Pharmacokinetics and hemostatic effect of different factor VIII-von Willebrand factor concentrates in von Willebrand's disease type III. *Ann Hematol* 1992;65:253-259
 91. Menache D, Aronson DL, Darr F, Montgomery RR, The Cooperative Study Group. Pharmacokinetics of von Willebrand factor and factor VIIIc in patients with severe von Willebrand disease (type 3): estimation of the rate of factor VIIIc synthesis. *Br J Haematol* 1996;94:740-745
 92. Ver Elst K, Van Vliet HHD, Kappers-Klunne MC, Leebeek FWG. In vitro studies, pharmacokinetic studies and clinical use of a high purity double virus inactivated FVIII/VWF concentrate (Immunate) in the treatment of von Willebrand disease. *Thromb Haemost* 2004;92:67-74
 93. Auerswald G, Eberspächer B, Engl W, et al. Successful treatment of patients with von Willebrand disease using

- a high-purity double-virus inactivated factor VIII/von Willebrand factor concentrate (Immunate). *Semin Thromb Hemost* 2002;28:203–213
94. Mannucci PM, Chediak J, Bymes W, et al. Treatment of von Willebrand disease with high-purity factor VIII/von Willebrand factor concentrate: a prospective, multicenter study. *Blood* 2002;99:450–456
 95. Michiels JJ, Berneman ZN, Van Der Planken M, Schroyens W, Budde U, Van Vliet HHDM. Bleeding prophylaxis for major surgery in patients with type 2 von Willebrand disease with an intermediate purity factor VIII-von Willebrand factor concentrate (Haemate-P). *Blood Coagul Fibrinolysis* 2004;15:323–330
 96. Mannucci PM, Federici AB. Management of inherited von Willebrand disease. *Baillieres Clin Haematol* 2001;14:455–462
 97. Mannucci PM. Treatment of von Willebrand's Disease. *N Engl J Med* 2004;351:683–694
 98. Rodeghiero F, Castaman G. Treatment of von Willebrand disease. *Semin Hematol* 2005;42:29–35
 99. Pasi KJ, Collins PW, Keeling DM, et al. Management of von Willebrand disease: a guideline from the UK Haemophilia Centre Doctor's Organization. *Haemophilia* 2004;10:218–231
 100. Gill JC, Ewenstein BM, Thompson AR, Mueller-Veltens G, Schwartz BA. The Humate-P Study Group. Successful treatment of urgent bleeding in von Willebrand disease with factor VIII/VWF concentrate (VWF:RC₀) to measure potency and to guide therapy. *Haemophilia* 2003;9:688–695
 101. Thompson AR, Gill JC, Ewenstein BM, Mueller-Veltens G, Schwartz BA. Successful treatment for patients with von Willebrand disease undergoing urgent surgery using factor VIII/VWF concentrate (Humate-P). *Haemophilia* 2004;10:42–51
 102. Goudemand J, Mazurier C, Marey A, et al. Clinical and biological evaluation in von Willebrand's disease of a von Willebrand factor concentrate with low factor VIII activity. *Br J Haematol* 1992;80:214–221
 103. Menache D, Aronson DL, Darr F, Montgomery RR. The Cooperative Study Group. Pharmacokinetics of von Willebrand factor and factor VIIIIC in patients with severe von Willebrand disease (type 3): estimation of the rate of factor VIII synthesis. *Br J Haematol* 1996;94:740–745
 104. Menache D, Aronson DL. New treatments of von Willebrand disease: plasma derived von Willebrand factor concentrates. *Thromb Haemost* 1997;78:566–570
 105. Goudemand J, Negrier C, Ounmoughene N, Sultan Y. Clinical management of patients with von Willebrand's disease with a VHP VWF concentrate: the French experience. *Haemophilia* 1998;4(suppl 3):48–52