

## Current Management of von Willebrand Disease

a report by

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Von Willebrand disease is the most common inherited bleeding disorder and is caused by quantitative (VWD1 and VWD3) or qualitative (VWD2) defects of von Willebrand factor (VWF). Inherited by autosomal dominant or recessive patterns, women with mild forms of VWF are more symptomatic.<sup>1</sup> In population-based studies the prevalence of VWD is high (0.81%), but the clinical relevance of many of these cases is uncertain.<sup>2</sup> Taking patients referred for clinical manifestations of bleeding, the actual prevalence is 66–100 cases per million of the general population.

The up-to-date classification of VWD has proposed six different types: VWD1, VWD3, VWD2A, VWD2B, VWD2M and VWD2N.<sup>3</sup> A partial quantitative defect marks VWD1. VWD3 is characterised by the nearly total absence of VWF in plasma and platelets. VWD1 is easily distinguished from VWD3 by milder VWF deficiency (usually in the range of 10–30U/dl), the autosomal dominant pattern of inheritance and the presence of a milder bleeding tendency.<sup>3</sup>

In the past VWD1 was reported to be the most frequent form of VWD, accounting for approximately 70% of cases. A 2008 study based on the reappraisal of diagnoses of VWD1 after 10 years in 1,234 patients followed by 16 Italian centres has established that VWD1 accounted for only 671 of the 1,234 cases (55%). Many cases previously diagnosed as VWD1 were re-diagnosed as VWD2 due to discrepant VWF measurements (ratio of ristocetin co-factor activity [VWF: RCo] to VWF: Ag <0.7).<sup>4</sup> The ages and VWD type distribution of the 1,234 patients enrolled on the registry are shown in *Figure 1*. The presence of qualitative defects of VWF in previously diagnosed VWD1 has also been reported in 154 families evaluated prospectively by the European study.<sup>5</sup> Four types of qualitative defects of VWF have been identified, reflecting different pathophysiological mechanisms.<sup>3</sup> VWD2A and VWD2B are marked by the absence of high-molecular-weight VWF multimers in plasma. In VWD2B there is also an increased affinity of VWF for its platelet receptor, glycoprotein Ib-alpha (GPIb $\alpha$ ). The identification of qualitatively abnormal variants with decreased platelet-dependent function and a normal multimeric structure marks VWD2M. VWD2N shows a full array of multimers, the defect being in the N-terminal region of the VWF where the binding domain for FVIII is located. This type is phenotypically distinguishable from mild haemophilia-A only by the abnormal binding of FVIII to VWF (VWF: FVIIIb).

### Clinical and Laboratory Diagnosis

Three main criteria are required for the correct diagnosis of VWD: positive bleeding history since childhood; reduced VWF activity in plasma; and a history of bleeding in the family with autosomal dominant or recessive inheritance. Evidence-based diagnosis of VWD1 has recently been proposed according to these three criteria.<sup>6</sup>

Clinical manifestations include excessive mucocutaneous bleeding and prolonged 'oozing' after surgical procedures. In women menorrhagia may be the only clinical manifestation. Soft-tissue and joint bleeding is rare, except in patients with VWD3 that is characterised by severe deficiencies of VWF and FVIII. In most patients with VWD1 the clinical expression of the disease is usually mild. The severity of the clinical expression of the condition increases in both VWD2 and in particular in VWD3. The severity of bleeding generally correlates with the degree of reduction of VWF: RCo and FVIII. Few detailed descriptions of symptoms are available to date.<sup>4,7,8</sup>

*Table 1* shows the relative frequency of bleeding symptoms in three large series of patients diagnosed at specialised centres. Several attempts were recently made to evaluate the sensitivity and specificity of bleeding symptoms, especially in the mild cases with VWD1 and VWF: RCo (>20U/dl). In a multicentre study carried out in obligatory carriers of VWD1, menorrhagia and epistaxis were poor predictors of the disease, while cutaneous bleeding and bleeding after dental extractions were more sensitive symptoms for diagnosis.<sup>9</sup> A bleeding severity score (BSS) has been analysed in affected and non-affected members of 154 families enrolled prospectively in a large European study on VWD1.<sup>10</sup> Despite the fact that this BSS was investigated prospectively in VWD1, this approach can be useful in all VWD, as was recently proposed in a prospective study.<sup>11</sup>

The diagnosis of VWD, particularly VWD1, may require several laboratory tests to be repeated on different occasions. These tests are usually applied for patients with suspected bleeding disorders. *Table 2* summarises the different steps for VWD diagnosis. The bleeding time (BT), an original hallmark of the disease, is not always prolonged and may be normal in patients with mild forms of VWD, such as those with VWD1 and normal platelet VWF content.<sup>4</sup> It is therefore not particularly useful for diagnosis. Evaluation of closure time (CT) with the platelet function analyser (PFA-100) gives a rapid and simple measure of VWF-dependent platelet function at high shear stress. It can be performed in whole blood and can be employed instead of the BT in children or when the BT is not feasible. This system is sensitive and reproducible for VWD screening. The computerised axial tomography (CT) scan is normal in VWD2N and cannot be modified in VWD3 after the administration of VWF/FVIII concentrates.<sup>12</sup> Based on these observations, BT and CT were not introduced in the flow chart to be used in the differential diagnosis of VWD types (see *Figure 2*).

Molecular and pre-natal diagnoses of VWD have been in use since the early 1990s. The first mutations were found within exon-28 of the VWF gene that is responsible for domains A1, A2 and A3. Most VWD2A cases are due to missense mutations in the A1 domain, with

**Table 1: Prevalence (%) of Bleeding Symptoms in Patients with von Willebrand Disease from Different Cohorts and in Normal Individuals**

	Iranian		Italian*		Scandinavian	
Ethnicity VWD	Type 3	Type 1	Type 2	Type 3	VWD	Normal
Symptoms	(n=348)	(n=671)	(n=497)	(n=66)	(n=264)	(n=500)
Epistaxis	77	61	63	66	62	5
Menorrhagia	69	32	32	56	60	25
Post-extraction bleeding	70	31	39	53	51	5
Haematomas	NR	13	14	33	49	12
Bleeding from minor wounds	NR	36	40	50	36	0.2
Gum bleeding	NR	31	35	56	35	7
Post-surgical bleeding	41	20	23	41	28	1
Post-partum bleeding	15	17	18	26	23	19
Gastrointestinal bleeding	20	5	8	20	14	1
Joint bleeding	37	3	4	45	8	0
Haematuria	1	2	5	12	7	1
CNS bleeding	NR	1	2	9	NR	0

CNS = central nervous system; NR = not reported.

\*Bleeding symptoms in Italian patients have recently been re-calculated according to the updated results of the Italian registry of VWD and are different from those previously reported.<sup>11</sup>

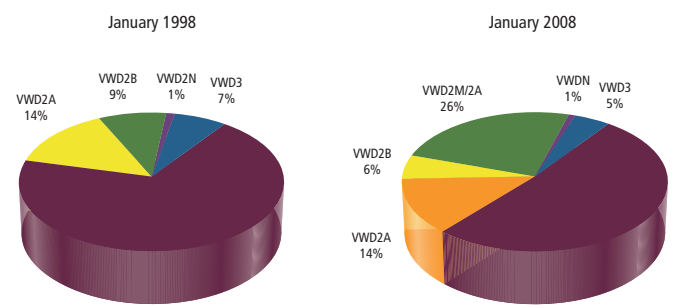
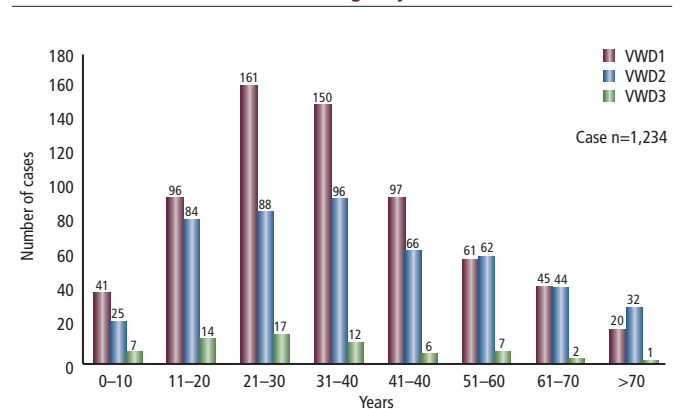
Adapted from Federici,<sup>4</sup> Silver<sup>7</sup> and Lak et al.<sup>8</sup>

R1597W, Q, Y and S1506L accounting for about 60% of them.<sup>13</sup> Similarly, the majority of VWD2B cases are also due to missense mutations in the A1 domain, about 90% being caused by R1306W, R1308C, V1316M and R1341Q.<sup>13</sup> A few heterogeneous mutations, also located within the A1 domain, underlie VWD2M. A recurrent mutation in VWD1/2M Vicenza, R1205H, has been identified in families from Europe. Another mutation, M740I, is seen exclusively in families from the Vicenza area in the north-east of Italy.<sup>14,15</sup> Missense mutations in the FVIII-binding domain at the amino-terminal portion of VWF are responsible for VWD2N.<sup>16</sup> The molecular defects responsible for VWD2 are located in specific VWF domains (see Figure 3). Mutations responsible for VWD1 and VWD3 are spread throughout the entire VWF gene.

The genetic causes of VWD1 are still elusive in many cases, especially in those with a mild phenotype. More information on the molecular basis of VWD1 has been collected by two multicentre international studies. In the European study, recruitment was based on the historical diagnosis of VWD1, which included 278 affected cases and 312 non-affected family members.<sup>5</sup> The Canadian investigators recruited 123 families for which the index case had bleeding symptoms and VWF levels between 5 and 50U/dl.<sup>17</sup>

In this study, subjects with abnormal multimeric patterns, or other evidence of qualitative defects, were excluded. The most important conclusions from both studies were: despite the selection of patients based on bleeding history, candidate VWF mutations were not found for 27% (Canadian) and 36% (European) of index cases diagnosed with VWD1; and the spectrum of VWD1 mutations was different from that found in VWD3. VWD1 is therefore not at all like heterozygous VWD3, as VWF defects that occur in VWD1 are usually caused by dominant VWF abnormalities that affect VWF secretion or clearance without altering multimeric patterns or platelet binding.

In VWD3, partial or total gene deletions have been reported.<sup>18</sup> Homozygosity for gene deletion may be associated with the appearance of allo-antibodies against VWF, which may render replacement therapy ineffective and stimulate anaphylactic reactions to treatment.<sup>19,20</sup>

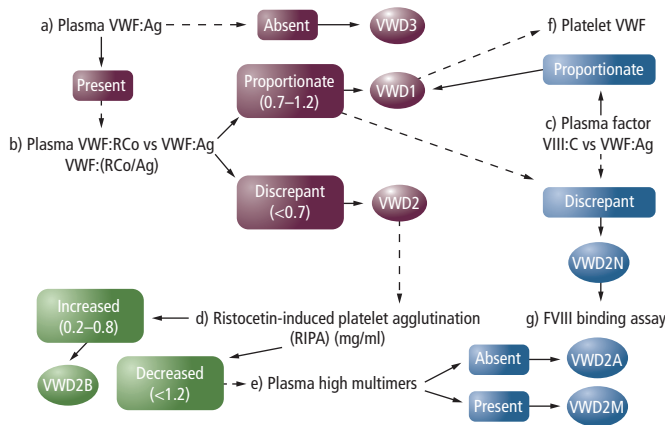
**Figure 1: Age Distribution of the 1,234 Patients Enrolled in the Italian von Willebrand Disease Registry**

Each decade reports the frequency of VWD1, VWD2 and VWD3 (upper panel). VWD-type distribution of the same population, as classified in 2008 according to the recommendations reported in reference 4; note that the percentage of VWD1 is lower than that found 10 years before by the Italian Association of Haemophilia Centres (lower panel).

Mutations may be scattered over the entire gene, but some (e.g. 2680delC or Arg2535X) are particularly recurrent in northern Europe.<sup>21</sup>

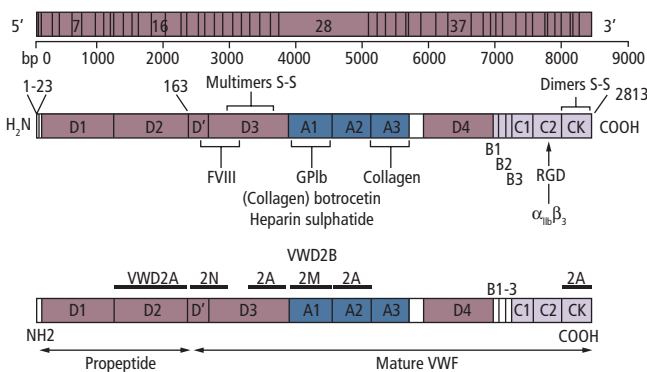
Gene defects of VWD3 patients from three different populations have now been studied. There was no 'founder' effect and mutations were distributed throughout the entire VWF gene.<sup>22</sup> Compared with haemophilia, most VWD patients show relatively mild bleeding symptoms. Pre-natal diagnosis is required in the cases of parents

**Figure 2: Flow Chart Proposed for the Diagnosis of Different von Willebrand Disease Types**



VWD3 can be diagnosed in case of immeasurable VWF: Ag (a). A proportionate reduction of both VWF: Ag and VWF: RCo with an RCo/Ag ratio >0.7 suggests type one VWD (b). If the VWF: RCo/Ag ratio is <0.7 type two is diagnosed. VWD2B (d) can be identified in cases of heightened RIPA (<0.8mg/ml), whereas types 2A and 2M cause low RIPA (>1.2mg/ml). Multimeric analysis in plasma (e) is necessary to distinguish between VWD2A (lack of the largest and intermediate multimers) and VWD2M (all of the multimers present). VWD2N can be suspected in cases of discrepant values for FVIII (c) and VWF: Ag (ratio <0.5), and diagnosis should be confirmed by the specific test (g) of VWF: factor VIII binding capacity (VWF: FVIIIb). In VWD1, the ratio between factor VIII and VWF: Ag is always >1 and the severity of VWD1 phenotype can usually be evaluated from platelet VWF (f) measurements. This figure is derived from that originally reported.<sup>4</sup>

**Figure 3: Schematic Representation of the von Willebrand Disease Gene Located in Chromosome 12**



The main exons are indicated with the number of base pairs from 5' to 3' (upper panel). The structure of VWF functional domains: the pre-pro-VWF is indicated with amino acids numbered from the amino- (aa 1) to carboxy-terminal portions (aa 2813) of VWF. Note the important C1 and D3 domains for formation of VWF dimers and multimers. The native mature subunit of VWF, after the cleaving of the pre-pro VWF, is described with its functional domains: the VWF binding sites for factor VIII (D' and D3), GPIIb, botrocetin, heparin, sulphatide, collagen (A1), collagen (A3) and the RGD sequence for binding to  $\alpha_{IIb}\beta_3$  (intermediate panel). Distribution of VWF mutations in patients with VWD type 2: the positions of mutations causing VWD2A, VWD2B, VWD2M and VWD2N are indicated with black bars throughout the VWF domains (lower panel).

already known to be carriers of VWD3 and with gene defects identified in an affected child. Since young children with VWD3 might carry deletions of the VWF gene that predispose to the allo-antibodies to VWF, every new child with VWD3 should be intensively investigated by searching deletions before starting extensive therapy with exogenous VWF concentrates.

**Treatment and Prevention of Bleeding**

The goal of treatment is to correct the dual defects of haemostasis – abnormal platelet adhesion due to low or defective VWF and abnormal intrinsic coagulation due to low FVIII.<sup>23</sup> Two main therapeutic approaches are available: desmopressin (DDAVP), which releases

**Table 2: Clinical and Laboratory Parameters Used for von Willebrand Disease Diagnosis**

<b>Patients at Risk of VWD</b>	
Clinical history – lifelong mucocutaneous and post-operative bleeding, to be collected with appropriate questionnaires to calculate the bleeding severity score (BSS).	
Screening tests – prolonged bleeding time (maybe normal); normal platelet count; and prolonged PTT (maybe normal).	
<b>Diagnosis and Definition of VWD Type</b>	
VWF antigen (a)	
VWF: ristocetin co-factor activity (b)	
Factor VIII (c)	
VWF multimeric structure on low-resolution gels (e)	
<b>Characterisation of VWD Type</b>	
Ristocetin-induced platelet agglutination (RIPA) (d)	
VWF multimeric structure on high-resolution gels (e)	
Platelet VWF content (f)	
Factor VIII binding assay (g)	

For the use of these tests see the diagnostic flow-chart reported in Figure 3 and also in reference 4.

endogenous VWF from endothelial cells; and exogenous VWF contained in VWF/FVIII concentrates. The choice of the two approaches related to VWD type is summarised in Table 3.

**Desmopressin**

Desmopressin (1-deamino-8-D-arginine vasopressin, DDAVP) is a synthetic analogue of vasopressin that is relatively inexpensive and carries no risk of transmitting blood-borne infectious agents. DDAVP, infused intravenously at a dose of 0.3µg/kg and diluted in 50ml of saline over 30 minutes, usually increases plasma VWF and FVIII three to five times above baseline levels within 30–60 minutes. The high VWF and FVIII levels generally last for six to eight hours.<sup>24</sup> As the responses in a given patient are consistent on different occasions, a test dose of DDAVP at the time of diagnosis is recommended to establish the individual response patterns.<sup>4</sup>

The protocols of the infusion test, with clinical and laboratory parameters to be measured, have been previously reported.<sup>25,26</sup> The criteria for biological responses are shown in Table 4. DDAVP infusions can be repeated every 12–24 hours depending on the type and severity of the bleeding episode. Most patients who are repeatedly treated become less responsive to therapy.<sup>27</sup> The drug is also available in concentrated forms for subcutaneous and intranasal administration. This can be convenient for home treatment.

Despite the widespread use of DDAVP in the treatment of VWD, there are no prospective clinical studies on its efficacy and safety along with determining its benefits and limits. A recent investigator-driven prospective study will correlate biological response with the clinical efficacy of DDAVP in more than 200 patients with VWD1 and VWD2.<sup>28</sup> Side effects of DDAVP are usually mild tachycardia, headache and flushing. These are attributed to the vasomotor effects of the drug and can often be attenuated by slowing the rate of infusion. Hyponatraemia and volume overload, due to the antidiuretic effects of DDAVP, are relatively rare and usually occur only in young children.<sup>29</sup>

Although no thrombotic episodes have been reported in VWD patients treated with DDAVP, this drug should be used with caution in elderly

patients with atherosclerotic disease and hypertension, further to reported side effects in patients with mild haemophilia A.<sup>30,31</sup>

### VWF/FVIII Concentrates

VWF/FVIII concentrates are indicated in VWD3, in VWD2B because DDAVP can induce transient thrombocytopenia and in all VWD1 or VWD2 patients who are not responsive to DDAVP or who may have contraindications to its use (see *Table 3*). Minimal requirements for plasma-derived VWF/FVIII concentrates in VWD management are: they must contain VWF and some FVIII; they should be treated by virucidal methods; and before clinical use they should be tested for pharmacokinetics (PK) and efficacy in retrospective or prospective clinical trials in relatively large numbers of VWD patients.<sup>23</sup> Among the many VWF/FVIII concentrates available, only a few can meet these requirements (see *Table 5*). They can be given to stop bleeding episodes when they occur (treatment on demand), to prevent bleeding during surgery (prophylaxis for surgery) and to prevent recurrent bleeding at specific sites (secondary long-term prophylaxis).

The PK and clinical efficacy results of the first prospective study in VWD were published in 2002.<sup>32</sup> This study included 53 patients receiving treatment with a double virus-inactivated VWF/FVIII concentrate (Alphanate®) for 87 bleeding episodes and in 39 patients receiving treatment prior to 71 surgical or invasive diagnostic procedures. A good clinical response was observed in 86% of the spontaneous bleeding episodes and in 71% of the surgical or invasive procedures.<sup>32</sup> Two retrospective studies and one prospective study have also been performed using Fandhi®, a concentrate manufactured using a process very similar to that of Alphanate.<sup>33–35</sup>

Haemate-P® or Humate-P®, an intermediate-purity VWF/FVIII concentrate, has been widely used in VWD. This product was introduced into clinical practice in Europe in 1984 (Haemate-P) and in the US in 1999 (Humate-P). The first PK study of Haemate-P, published in 1998, was a single-centre evaluation involving six VWD3 patients.<sup>36</sup> The result of a large retrospective study organised by the Canadian haemophilia centres was published in 2002.<sup>37</sup> Other published studies include two retrospective analyses of Haemate P efficacy and safety in Italian VWD patients,<sup>38,39</sup> along with two prospective, multicentre, open-label, non-randomised studies conducted in the US on Humate-P used in urgent bleeding and surgical events.<sup>40,41</sup> The results of another prospective study in elective surgery using HaemateP/Humate-P with dosing based on PK have recently been published.<sup>42</sup>

Wilate® has been used in Germany since 2005 for VWD management.<sup>43</sup> The results of its efficacy and safety in acute bleeding episodes, surgical interventions and secondary long-term prophylaxis will be published within a few months. Data on the PK and clinical efficacy of Biostate®, a VWF/FVIII concentrate available in Australia and Asia, have also been reported.<sup>44,45</sup> A plasma-derived VWF concentrate with low FVIII levels was introduced in France in 1992 and the first PK study in VWD3 was published in 1996.<sup>46</sup> An improved version of this concentrate (Wilfactin®) that is almost devoid of FVIII was evaluated in two large French and European studies and data on PK has been already published.<sup>47</sup> Results in VWD3 show no major differences in VWF:RCo and VWF:Ag for the concentrates that did or did not contain FVIII. The only difference was an approximate six-hour delay in FVIII increase with the concentrate devoid of FVIII. Administration of exogenous FVIII is recommended in VWD3 cases of acute life-threatening

**Table 3: Treatment of Different Types of von Willebrand Disease**

	Treatment of Choice	Alternative and Adjunctive Therapy
VWD1	Desmopressin	Antifibrinolytics, oestrogens
VWD2A	VWF/FVIII concentrates	Desmopressin for mild bleeding
VWD2B	VWF/FVIII concentrates	
VWD2M	Desmopressin	VWF/FVIII concentrates
VWD2N	Desmopressin	VWF/FVIII concentrates
VWD3	VWF/FVIII concentrates	Desmopressin, platelet concentrates
VWD3 with alloantibodies	Recombinant factor VIII	Recombinant activated factor VII

**Table 4: Recommendations for the Infusion Test with DDAVP<sup>4,25,26</sup>**

Infusion protocol	Administer in 30 minutes 0.3µg/kg of desmopressin in 50ml of saline. The same dosage can also be administered subcutaneously.
Clinical and laboratory parameters	Factor VIII/VWF activities must be measured before and 0.5, 1, 2 and 4 hours after the administration of Desmopressin. Check platelet count before and at least 2 hours after infusion.
Definition of responsiveness	VWD patients should be considered responsive to desmopressin if after 2 hours they show an increased baseline level of FVIII: C and VWF: RCo by at least three times, with levels of at least 30U/dl.

bleeding episodes or emergency surgeries.<sup>47</sup> The clinical efficacy results of the French and European studies have recently been reported.<sup>48</sup>

There is no evidence from either retrospective or prospective clinical studies that the six VWF/FVIII concentrates (Alphanate, Biostate, Fandhi, Haemate-P/Humate-P, Wilate, Wilfactin), reported in *Table 5*, differ with regards to efficacy, as no head-to-head clinical study has been carried out. All of these VWF/FVIII concentrates can be effective to manage or prevent bleeding in patients with VWD.

### Treatment of Patients with Allo-antibodies to von Willebrand Factor

For the rare patients with VWD3 who develop anti-VWF allo-antibodies after multiple transfusions, the use of VWF/FVIII concentrates not only is ineffective, but also may even cause post-infusion anaphylaxis due to the formation of immune complexes.<sup>19,49</sup> These reactions may be life-threatening. To overcome this drawback, a patient undergoing emergency abdominal surgery was treated with recombinant FVIII. This product contains no VWF and could not cause anaphylactic reactions. In view of the very short half-life of FVIII without its VWF carrier, recombinant FVIII had to be administered by continuous intravenous infusion at very large doses to keep FVIII levels above 50U/dl for 10 days after surgery.<sup>49</sup>

Another possible therapeutic approach is recombinant-activated factor VII (rFVIIa), which can be used in VWD patients with allo-antibodies according to the same dosage and regimens as for haemophilia-A patients with inhibitors.<sup>50,51</sup>

### Secondary Long-term Prophylaxis

Patients with severe forms of VWD may have frequent haemarthroses, especially when FVIII levels are below 10U/dl. Some of them develop target joints like patients with moderate hemophilia-A. Some also have

**Table 5: Plasma-derived Concentrates Containing von Willebrand Factor**

A. Concentrates with Published Activity in VWD Subjects						
Concentrate	Purification Procedures	Virucidal Rx	VWF: RCo/Ag	VWFRCo/FVIII	Available in	Manufacturer
Alphanate	Heparin ligand CT	SD; dry heat	0.6	1.2	GE, IT, UK, US	Grifols (US)
Biostate	Precipitation/heparin ligand CT	SD; dry heat	0.8	2.0	AU, ASIA	CslBehring
Fanahi	Precipitation/heparin ligand CT	SD; dry heat	0.6	1.6	SP-IT	Grifols (Sp)
Haemate-P <sup>†</sup>	Polyelectrolyte precipitations	Pasteurisation	0.8	2.5	ASIA, EUR, US	CslBehring
Wilate	Affinity CT, size exclusion	SD; dry heat	0.7	0.8	GE	Octapharma
Wilfactin	Ion-exchange, affinity CTs	SD; NF; dry heat	0.7	60	FR	LFB (Lille)
B. Concentrates with Limited Activity or No Published Studies in VWD Subjects						
Emoclot	Ion-exchange CT	SD; dry heat	0.5	1.2	BR, IT	Kedrion
Immunate	Ion-exchange CT	SD; vapour heat	0.2	0.2	EUR	Baxter
8Y	Heparin/glycine precipitations	Dry heat	0.3	0.8	UK	BioProducts

CT = chromatography; SD = solvent-detergent (t-N-butyl-PO4 with polysorbate, Tween or octoxynol, Triton); nf = nanofiltration; na = not applicable (multiple countries).

\* Ratio of ristocetin co-factor activity (VWF: RCo) to VWF: Ag or FVIII activity expressed as IU/ml (or % of a normal pool).

<sup>†</sup> Humate P in US.

recurrent gastrointestinal (GI) bleeding, often without lesions in the GI tract, and need treatment every day or every other day. There are children who have epistaxis frequently and severely enough to cause anaemia. In these frequent and severe bleeders, the optimal therapy may be regular prophylaxis with VWF concentrates rather than 'on-demand treatment' on the occasion of bleeding episodes.

The largest report on secondary prophylaxis in VWD was conducted in Sweden in 35 patients with severe forms of VWD.<sup>52</sup> Secondary prophylaxis was also implemented in a cohort of Italian patients with VWD.<sup>53</sup> These two retrospective studies suggest that cost-effectiveness of these prophylaxis regimens versus on-demand therapy should be further evaluated in larger prospective studies.

### Future Perspectives

Current treatments available for VWD are satisfactory. For patients unresponsive to DDAVP, VWF/FVIII concentrates are the only form

of available treatment. The fact that they are fractionated from plasma is of concern for some, even if more than one viral inactivation method is used for most concentrates in the manufacturing process. Haemate P/Humate P is the only concentrate that uses only one viral inactivation method (pasteurisation), and the safety record of this product is impeccable.

Despite this favourable situation, there are advanced plans to develop a therapeutic preparation of recombinant VWF. The product containing only VWF will require the concomitant administration of FVIII for the control of acute bleeding episodes and for the prevention of excessive bleeding at the time of major surgery. Attempts to partially correct VWD through gene replacement therapy are also in progress.<sup>54</sup> ■

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