Guidelines for the diagnosis and management of von Willebrand disease in Italy

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Summary. von Willebrand disease (vWD) is a bleeding disorder caused by quantitative (type 1 and 3) or qualitative (type 2) defects of von Willebrand factor (vWF). The molecular basis of type 2 and 3 vWD are now known and those of type 1 vWD are being understood. Phenotypic diagnosis is based on the measurements of plasma and platelet vWF, of the ability of vWF to interact with platelet receptors and the analysis of the multimeric structure of vWF. Due to the heterogeneity of vWF defects and the variables that interfere with vWF levels, a correct diagnosis of types and subtypes may sometimes be difficult but is very important for therapy. The aim of treatment is to correct the dual defects of haemostasis, i.e. abnormal intrinsic coagulation expressed by low levels of factor VIII (FVIII) and abnormal platelet adhesion. Desmopressin is the treatment of choice in patients with type 1 vWD, who account for approximately 70% of cases, because it corrects FVIII–vWF levels and the prolonged bleeding time (BT) in the majority of these patients. In type 3 and in severe forms of type 1 and 2 vWD patients, desmopressin is not effective and it is necessary to resort to plasma concentrates containing FVIII and vWF. Treated with virucidal methods, these concentrates are effective and safe, but they cannot always correct BT defect. Platelet concentrates or desmopressin can be used as adjunctive treatments when poor correction of BT after plasma concentrate treatment is associated with continued bleeding.

Keywords: congenital von Willebrand disease, desmopressin, factor VIII/ von Willebrand factor concentrates, genetic and molecular diagnosis, von Willebrand factor.

Introduction

von Willebrand disease (vWD) is a bleeding disorder caused by a quantitative or qualitative defect of von Willebrand factor (vWF), the high molecular weight glycoprotein that plays an essential role in the early phases of haemostasis by promoting platelet adhesion to the subendothelium, and platelet aggregation under high shear stress conditions [1]. As vWF is also the carrier of factor VIII (FVIII) in plasma, deficiency of vWF also results in an impairment of intrinsic blood coagulation. In the majority of cases, vWD is congenital, inherited in an autosomal dominant fashion; an autosomal recessive inheritance is also described in some cases. Patients with vWD may have a mild, moderate or severe bleeding tendency since childhood, usually proportional to the degree of the vWF defect. Patients with a negative family history and with a recent personal history of bleeding may have an acquired disease similar to congenital vWD; this is usually associated with other clinical conditions and is called acquired von Willebrand Syndrome (AvWS) [2,3]. In this article, the guidelines on diagnosis and treatment of vWD approved by the Members of the Italian Association of Hemophilia Centers (AICE) are presented.

Biosynthesis, structure and function of vWF

vWF is synthesized in endothelial cells and either stored in intracellular organelles known as Weibel–Palade bodies or secreted constitutively. It is also synthesized in megakaryocytes and stored in platelet alpha-granules (4). The gene coding for vWF is
178,000 bases long, is located on chromosome 12 and contains 52 exons. vWF is synthesized as a large precursor protein (360 kDa, 2813 amino acids), which consists of a 22-amino acid signal peptide, a pro-polypeptide (100 kDa, 741 amino acids) also known as vWF antigen II, and a mature subunit (270 kDa, 2050 amino acids). Dimers are formed in the endoplasmatic reticulum by covalent dimerization of the subunits at their C-termini. Multimers are formed in the Golgi apparatus and the secretory vesicles by covalent multimerization of these dimers at the N-terminus (Fig. 1). The pro-peptide of vWF is required for normal multimer formation and is cleaved off by furin, a dibasic paired amino acid-cleaving enzyme. vWF is released from endothelial cells as ultralarge multimers and circulates in the plasma as a series of multimers of very high molecular weight (500–20,000 kDa). Proteolysis is involved in the generation of these multimers, and shear stress enhances the susceptibility to proteolytic cleavage. About 1% of plasma vWF contains the pro-peptide, possibly due to incomplete processing. Recently, it was shown that human plasma contains a vWF-degrading enzyme, vWF-cleaving protease (vWF-CP) and that the cleavage site of this enzyme is located between amino acid residues 842Thr and 843Met in the A2 domain of the vWF subunit. vWF-CP cleaves ultralarge multimers, normally stored in Weibel–Palade bodies of vascular endothelium, from which they are secreted luminally into plasma and abluminally into the subendothelium. Each vWF subunit shows a characteristic pattern of homologous A, B, C and D domains, which are independent building blocks in many other proteins. The pro-peptide contains a D1 and D2 domain. The mature subunit consists of D’–D3–A1–A2–A3–D4–B1–B2–B3–C1–C2 domains, and a C-terminal part of 151 amino acids that has no internal homology (Fig. 1). vWF acts as an adhesive glycoprotein and mediates platelet adhesion to subendothelium through its binding sites for the platelet receptor GpIb–α–IX and collagen, and platelet–platelet interactions through its binding site for platelet GpIIb/IIIa. Additional binding sites are those for heparin and sulphatides. Apart from its adhesive functions, vWF serves as a carrier protein for FVIII. By the noncovalent interaction between the two proteins, FVIII is protected against binding to membrane surfaces and to proteolytic attack by a variety of serine proteases, including activated protein C (5).

Pathophysiology and classification

Inherited vWD has been subdivided into three types, which reflect its pathophysiology. Type 1 and 3 vWD reflect, respectively, the partial or virtually complete deficiency of vWF while type 2 vWD reflects a qualitative deficiency of vWF. The revised classification introduced in 1994 by Sadler [6], recommends that type 2 vWD is subdivided into four subtypes (2A, 2B, 2M, 2N) according to specific details of the phenotypic features (Table 1). This classification also addresses the problems of compound heterozygosity and of disorders related to vWD that are not due to a defect in the vWF gene, such as ‘platelet-type/pseudo-vWD’ and AvWS.

![Fig. 1. Domain structure and processing of von Willebrand factor (vWF). The domain structure (A–D) of prepro-vWF and mature vWF are shown. In the endoplasmic reticulum, carboxy-terminal disulphide-bonded dimers are formed. Disulphide bonds that result in multimerization occur late in the secretory pathway. The two platelet receptors, Gp Ib and αIIb β3, are indicated. Other molecular interactive site are also depicted.](image-url)
Prevalence

vWD is the most frequent inherited bleeding disorder. Nilsson [7] reported a frequency of approximately 125 cases per million in Sweden, twice as frequent as for haemophilia. Rodeghiero et al. [8], during a large Italian epidemiological study in children, found the prevalence to be 0.82%. More recent studies in different populations confirm a prevalence of the disease of approximately 1–2% [9,10]. Among the different vWD types, type 1 is the most frequent (60–80%); all type 2 vWD variants are 15–30%, while type 3 is diagnosed in 5–10% of vWD patients [11–15]. For example, in the 1286 vWD patients enrolled into the Italian National Registry of vWD (ReNaWi), the actual distribution 73% type 1, 21% type 2, and 6% type 3 [16]. The prevalence of different vWD subtypes as reported in the literature is shown in Table 2.

Clinical and phenotypic diagnosis

The diagnostic workup of vWD occurs in three steps: (i) identification of patients suspected for vWD, on the basis of the clinical history and results of the screening tests of haemostasis; (ii) diagnosis of vWD with indication of its type; and (iii) characterization of the subtype (Table 3).

Clinical history and screening tests

Clinical history. vWD should be suspected in patients with a history of mucocutaneous and postoperative bleeding, especially if family history suggests an autosomal pattern of inheritance. The most common symptoms associated with vWD are epistaxis, bleeding after dental extractions, and menorrhagia. The bleeding tendency, however, is highly variable and depends on the type and severity of the disease. In many patients with type 1 or type 2 vWD, the bleeding tendency may be mild or absent. In contrast, patients with type 3 vWD have a

Table 1. Classification of vWD.

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>vWD is caused by mutation of the vWF locus</td>
</tr>
<tr>
<td>2</td>
<td>Type 1 vWD refers to partial quantitative deficiency of vWF</td>
</tr>
<tr>
<td></td>
<td>Type 2 vWD refers to qualitative deficiency of vWF</td>
</tr>
<tr>
<td></td>
<td>Type 3 vWD refers to virtually complete deficiency of vWF</td>
</tr>
<tr>
<td>3</td>
<td>Type 2A vWD refers to qualitative variants with decreased platelet-dependent function that is associated with the absence of high-molecular-weight multimers</td>
</tr>
<tr>
<td>4</td>
<td>Type 2B vWD refers to qualitative variants with increased affinity for platelet GPIb</td>
</tr>
<tr>
<td>5</td>
<td>Type 2M vWD refers to qualitative variants with decreased platelet-dependent function not caused by the absence of high-molecular-weight multimers</td>
</tr>
<tr>
<td>6</td>
<td>Type 2N vWD refers to qualitative variants with markedly decreased affinity for factor VIII</td>
</tr>
</tbody>
</table>

From an updated version by Sadler [6].

Table 2. Frequency (%) of subtypes of vWD.

<table>
<thead>
<tr>
<th>Authors</th>
<th>No. of patients</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuddenham et al. [11]</td>
<td>134</td>
<td>75</td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td>Lenk et al. [12]</td>
<td>111</td>
<td>76</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Hoyer et al. [13]</td>
<td>116</td>
<td>71</td>
<td>23</td>
<td>6</td>
</tr>
<tr>
<td>Awidi [14]</td>
<td>65</td>
<td>59</td>
<td>30</td>
<td>11</td>
</tr>
<tr>
<td>Berliner et al. [15]</td>
<td>60</td>
<td>62</td>
<td>9</td>
<td>29</td>
</tr>
<tr>
<td>Federici et al. [16]</td>
<td>1286</td>
<td>73</td>
<td>21</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 3. Clinical and laboratory parameters used for vWD diagnosis.

Patients at risk for vWD

Clinical history: lifelong mucocutaneous and postoperative bleeding. Symptoms are sometimes present in other family members

Screening tests: prolonged bleeding time (maybe normal); normal platelet count; prolonged PTT (may be normal).

Diagnosis and definition of vWD type

vWF antigen
vWF: Ristocetin cofactor activity
Factor VIII
vWF multimeric structure on low resolution gels

Diagnosis of vWD subtype

Ristocetin-induced platelet agglutination (RIPA)
vWF multimeric structure on high resolution gels
Platelet vWF content
Factor VIII binding assay

For the use of these tests see the diagnostic flow-chart reported in Fig. 2.

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moderately severe haemorrhagic tendency; mucosal bleeding is very frequent and may be life-threatening. In addition, due to the severe FVIII defect, haematoma and haemarthrosis can occur. Postoperative haemorrhages are also common, especially in patients with very low FVIII levels. Data on the incidence of bleeding symptoms in vWD, as derived from three published reports [16–18], are summarized in Table 4. Because of the variability of the clinical history and the relatively low specificity of the bleeding symptoms, the diagnosis of vWD must rely also on the results of laboratory tests.

Screening tests. The platelet count is usually normal; mild thrombocytopenia may occur in patients with type 2B or Platelet-type/pseudo vWD. The bleeding time (BT) is usually prolonged, but may be normal in patients with mild forms of vWD such as those with type 1 and normal platelet content of vWF [19,20]. The prothrombin time (PT) is normal whereas the partial thromboplastin time (PTT) may be prolonged to a variable degree, depending on the plasma FVIII levels.

Diagnosis of vWD and identification of the type

vWF antigen (vWF:Ag), which can be measured in plasma by electroimmunoassay, immunoradiometric assay or a variety of enzyme-linked immunoabsorbent assays, is undetectable in type 3 vWD, whereas it may be low in type 1 and low or normal in type 2 vWD.

The assay for ristocetin cofactor activity (vWF:RCo) explores the interaction of vWF with the platelet glycoprotein Ib/IX/V complex and is still the standard method for measuring vWF activity. It is based on the property of the antibiotic ristocetin to agglutinate formalin-fixed normal platelets in the presence of vWF. In patients with a normal vWF structure (type 1 vWD), values of vWF:RCo are similar to those of vWF:Ag. Levels of vWF:RCo lower than those of vWF:Ag (ratio vWF:RCo/Ag < 0.7) are characteristic of type 2 vWD (see later, Fig. 2).

FVIII:C plasma levels are very low (1–5%) in patients with type 3 vWD. In patients with type 1 or type 2 vWD, FVIII may be decreased to a variable extent but is sometimes normal.

vWF can be analysed by agarose gel electrophoresis. Low-resolution agarose gels separate vWF multimers, which are conventionally indicated as high, intermediate and low molecular weight. In type 1 vWD all multimers are present, whereas in type 2 vWD, high and intermediate multimers are lacking.

Diagnosis of the subtype

For a diagnosis of patients with vWD and their correct treatment, other assays are necessary to define specific subtypes.

Ristocetin-induced platelet agglutination (RIPA) is measured by mixing different concentrations of ristocetin and patient platelet-rich plasma (PRP) in an aggregometer. Results are expressed as the concentrations of ristocetin (mg mL\(^{-1}\)) able to induce 30% of agglutination. Most vWD types and subtypes are characterized by hyporesponsiveness to ristocetin. Important exceptions are patients with type 2B vWD and Platelet-type/pseudo vWD, characterized by hyper-responsiveness to ristocetin due to a higher

<table>
<thead>
<tr>
<th>Bleeding symptoms</th>
<th>Type 1 (n = 944)</th>
<th>Type 2 (n = 268)</th>
<th>Type 3 (n = 74)</th>
<th>Type 3 (n = 348)</th>
<th>Scandinavian vWD patients (n = 264)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epistaxis</td>
<td>56</td>
<td>63</td>
<td>74</td>
<td>77</td>
<td>62</td>
</tr>
<tr>
<td>Menorrhagia</td>
<td>31</td>
<td>32</td>
<td>32</td>
<td>69</td>
<td>60</td>
</tr>
<tr>
<td>Bleeding after dental extraction</td>
<td>31</td>
<td>39</td>
<td>53</td>
<td>70</td>
<td>51</td>
</tr>
<tr>
<td>Hematomas</td>
<td>14</td>
<td>19</td>
<td>31</td>
<td>n.r.</td>
<td>49</td>
</tr>
<tr>
<td>Bleeding from wounds</td>
<td>36</td>
<td>40</td>
<td>50</td>
<td>n.r.</td>
<td>36</td>
</tr>
<tr>
<td>Gums bleeding</td>
<td>30</td>
<td>37</td>
<td>48</td>
<td>n.r.</td>
<td>35</td>
</tr>
<tr>
<td>Postoperative bleeding</td>
<td>20</td>
<td>23</td>
<td>41</td>
<td>41</td>
<td>28</td>
</tr>
<tr>
<td>Postpartum bleeding</td>
<td>17</td>
<td>18</td>
<td>26</td>
<td>15</td>
<td>23</td>
</tr>
<tr>
<td>Gastrointestinal bleeding</td>
<td>5</td>
<td>11</td>
<td>18</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>Petechiae</td>
<td>n.r.</td>
<td>n.r.</td>
<td>n.r.</td>
<td>n.r.</td>
<td>11</td>
</tr>
<tr>
<td>Joint bleeding</td>
<td>2</td>
<td>5</td>
<td>42</td>
<td>37</td>
<td>8</td>
</tr>
<tr>
<td>Hematuria</td>
<td>2</td>
<td>4</td>
<td>11</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>C.N.S. bleeding</td>
<td>0.5</td>
<td>2</td>
<td>8</td>
<td>n.r.</td>
<td>n.r.</td>
</tr>
</tbody>
</table>

Updated data from Federici et al. [16] compared with previous report by Silwer [17] and Lak et al. [18]. n.r., not reported.
than normal affinity of vWF for, respectively, platelet GP Ib/IX/V complex and platelet GPIb/IX/V.

vWF multimeric analysis with high resolution agarose gels allows better identification of type 1 and type 2 vWD subtypes [2,3].

Platelet vWF plays an important role in primary haemostasis, because it can be released from alpha granules directly to the site of vascular injury. On the basis of its measurement, type 1 vWD may be classified in three subtypes: type 1 'platelet normal', with a normal content of functionally normal vWF; type 1 'platelet low', with low concentrations of functionally normal vWF; and type 1 'platelet discordant', with normal concentrations of dysfunctional vWF [19,20].

FVIII binding assay measures the affinity of vWF for FVIII. In this assay, anti-vWF antibody is coated on wells of a microtitre plate and test plasma is added to the well. The FVIII/vWF complex from the plasma is bound by the antibody, following which FVIII is removed from the complex by a high ionic strength buffer. Excess recombinant FVIII (rFVIII) is then added and, after removal of unbound rFVIII, the vWF and bound rFVIII are assayed. This assay can permit type 2N vWD to be distinguished from mild to moderate haemophilia A because FVIII is abnormal in the former. Differential diagnosis of vWD subtypes can be performed by using the methods described above.

Diagnosis of type 3 vWD requires only the demonstration of no detectable values of vWF:Ag. Type 1 vWD is characterized by a proportionate reduction of both vWF:Ag and vWF:RCo with a RCo/Ag ratio > 0.7, while a type 2 vWD is present when the vWF:RCo/Ag ratio is < 0.7. According to the RIPA method, type 2B vWD is characterized by enhanced RIPA (< 0.8 mg mL\(^{-1}\)) while RIPA is always reduced (> 1.2 Mg mL\(^{-1}\)) in type 2A and
2M. Multimeric analysis in plasma is necessary to distinguish type 2A vWD (lack of the largest and intermediate multimers) from type 2M vWD (all the multimers are present as in normal plasma). Type 2N vWD can be suspected in case of discrepant values between FVIII and vWF:Ag (ratio < 1), and diagnosis should be confirmed by the specific test of vWF:FVIII binding capacity (vWF:FVIIIIB). In type 1 vWD, the ratio between FVIII and vWF:Ag is always ≥1 and the severity of type 1 vWD phenotype can usually be evaluated by performing platelet vWF measurements. A flow chart to be used in the diagnosis of vWD is reported (Fig. 2).

Additional tests used in the diagnosis of vWD include the closure time (CT) and assays of vWF activity based on binding to collagen (vWF:CB). The evaluation of CT with the PFA-100 (platelet function analyser) allows rapid and simple determination of vWF-dependent platelet function at high shear stress. This system has been demonstrated as being sensitive and reproducible for the screening of vWD, even though the CT is normal in type 2N vWD and remains abnormal in patients with type 3 vWD following the infusion of FVIII–vWF concentrates. The assays for vWF:CB are also available, and the ratio of vWF:CB and vWF:Ag levels appears to be useful to distinguish type 1 and 2 vWD [21]. Both assays are still not well standardized and are not officially approved by the Scientific Standardization Committee on vWF of the International Society of Thrombosis and Haemostasis.

**Type 1 vWD**

Type 1 is the most frequent form of vWD, inherited as an autosomal dominant trait in most cases; recessive transmission of type 1 vWD defects has also been shown [22,23]. Patients with type 1 vWD are characterized by mild to moderate bleeding symptoms, normal or variably prolonged BT and low levels of vWF:Ag, vWF:RCo and FVIII and normal multimeric structure. Definite diagnosis requires documentation of all three factors: inheritance, a history of bleeding, and low levels of normal vWF. Limitation of these criteria are the broad normal range for vWF levels and the variation in these levels over time [24–26]. Patients with type 1 vWD are very heterogeneous. As mentioned before, based on the platelet vWF content, three subtypes have been identified [19,20]. Patients with low levels of platelet vWF have more prolonged BT and usually more severe symptoms than those with normal platelet vWF, because they have also low levels of vWF stored in endothelial cells [27]. The diagnosis of type 1 vWD may also be complicated by several factors. ABO blood groups modify vWF levels in plasma [28]. vWF levels are higher in older individuals and in patients with diabetes. There is also evidence for hormonal regulation of vWF levels, which can be influenced by oestrogen and thyroid hormones and are significantly increased during pregnancy; vWF also appears to fluctuate in response to mild exercise [29]. A dominant type 1 vWD has been identified with mutated cysteine residues on the D3 domain of vWF [30].

**Type 2 vWD**

Type 2A is the most frequent subtype among type 2 vWD [31]. It is inherited mainly with an autosomal dominant pattern but a recessive pattern is also described [32]. Patients with 2A vWD are identified by normal to slightly reduced vWF:Ag levels and markedly low vWF:RCo, with an abnormal multimeric pattern characterized by loss of the high molecular weight multimers and increase of the intensity of low molecular weight multimers. Type 2A vWD are due to specific mutations located within the A2 domain of vWF subunit, and data obtained by expression studies show that two mechanisms are responsible for this defect [33]. One class of mutations, referred to as group 1, causes defective intracellular transport of vWF and impairs the assembly, storage and secretion of large vWF multimers in both plasma and platelets. Group 2 mutations do not interfere with vWF assembly or secretion, but render the multimers more sensitive to proteolysis in plasma [34]. Another cause of high molecular weight deficiency in type 2A vWD is a defective post-translational processing that includes defects of dimerization at the vWF C-terminus in the subtype previously described as type IID [35], and defects of further polymerization of vWF dimers to multimers in their N-termini. The latter multimerization defects can result either from mutations in the D1 and D2 domains of the vWF propeptide, which is necessary to catalyse intermolecular disulfide binding at the D3 domain of mature vWF in the subtype previously indicated as IID [36], and defects of further polymerization of vWF dimers to multimers in their N-termini. The latter multimerization defects can result either from mutations in the D1 and D2 domains of the vWF propeptide, which is necessary to catalyse intermolecular disulfide binding at the D3 domain of mature vWF in the subtype previously indicated as IIC, or from mutations in the D3 domain itself in the subtypes indicated as IIE and IIF as well as in type IIC Miami [35,36].

Type 2B can be identified because of heightened response to ristocetin and absence of large multimers from plasma [37]. The multimeric structure of platelet vWF and of vWF produced by cultured endothelial cells is normal [38]. The inheritance pattern is mainly autosomal dominant, but cases
with apparently recessive pattern have also been described [39]. A large degree of phenotypic heterogeneity has been identified since the original description. Typical features for 2B vWD are mild thrombocytopenia with increased mean platelet volume, prolonged BT, low to normal FVIII, low to normal vWF:Ag, low vWF:RCo and heightened RIPA. Thrombocytopenia can be more pronounced during pregnancy [40,41]. In some families, spontaneous platelet aggregation occurs [42,43]. More than 20 different missense mutations and one small insertion have been identified in type 2B, all located within the A1 domain of the vWF subunit, and the abnormal binding to platelet glycoprotein has been confirmed by expression studies in the majority of these mutants [33].

Type 2M (‘M’ for multimers) includes variants in which binding to platelets is impaired but the vWF multimeric distribution is normal. This phenotype may be produced by mutations that inactivate specific binding sites for ligands on platelets or collagens. Laboratory results generally are similar to those in type 2A, but there are high molecular weight forms. The type 2M mutations that have been characterized are located within domain A1 of the vWF subunit and show reduced binding to platelet GPIb in studies of expressed mutants [44–47]. Among this subgroup of vWD variants, patients with type 2M ‘Vicenza’ have low levels of vWF antigen but have larger than normal multimers (supranormal) in plasma, similar to those observed in plasma after desmopressin infusion and in endothelial cells and platelets [48]. Candidate missense mutation in type 2M ‘Vicenza’ have been identified in domain D3 [49].

Type 2N vWD (‘N’ for Normandy) is characterized by normal levels of vWF:Ag and vWF:RCo, and normal multimeric structure, but low plasma FVIII levels. It therefore resembles haemophilia A, but its inheritance pattern is not X-linked but autosomal recessive. Low FVIII levels, at variance with haemophilia A, are due to decreased plasma half-life of FVIII, which cannot bind to vWF as a consequence of an intrinsic abnormality of vWF [50,51]. Type 2N can be caused by several missense mutations, all localized to the D’ and D3 domains of vWF subunit. Co-inheritance of a type 2N mutation with a type 1 vWD allele may contribute to the variable expressivity of type 1 vWD [52].

**Type 3 vWD**

Type 3 (severe) vWD is caused by impaired biosynthesis of vWF and is characterized by undetectable levels of vWF in plasma and platelets. As vWF is also the carrier of FVIII, plasma levels of FVIII are very low (1–5%). As a consequence, patients with type 3 vWD have a severe bleeding tendency, characterized not only by mucocutaneous haemorrhages but also by haemarthroses and haematomas as observed in severe haemophilia. The inheritance pattern of type 3 vWD is autosomal recessive and its prevalence is 1–5 per million population [1–3]. Alloantibodies against vWF may arise in 5–8% of patients treated with FVIII–vWF concentrates [53,54]; gene deletions predispose to the formation of alloantibodies [55]. The presence of antibodies has been demonstrated in vitro by the capacity of patient plasma to inhibit RIPA of normal PRP in a time-dependent manner [53]. In vivo, the antibodies are responsible for a poor clinical response to replacement therapy. In some patients with high antibody titres, replacement therapy not only is ineffective but also may trigger life-threatening anaphylactic reactions [56,57]. The most common mutations in type 3 vWD are total or partial deletions, nonsense, splicing and frameshift mutations, found throughout the 52 exons of the vWF gene [58–62].

**Platelet-type/pseudo vWD**

Platelet-type or pseudo vWD is a primary platelet disorder, characterized by increased affinity of the platelet GP Ib/IX/V complex for normal vWF [63,64]. These patients have clinical and laboratory features similar to those of type 2B vWD: BT is prolonged, FVIII and vWF levels are variably reduced, RIPA is heightened, multimeric analysis reveals a deficiency of the high molecular weight multimers. The lack of high molecular weight multimers is probably due to increased in vivo utilization, secondary to heightened interaction between vWF (abnormal in 2B and normal in platelet-type vWD) and its receptor (normal in 2B and abnormal in platelet-type vWD). A way to distinguish platelet-type/pseudo vWD from 2B vWD is to add purified vWF to patient PRP in an aggregometer. In case of platelet-type/pseudo vWF, normal vWF will induce platelet aggregation, whereas it will not in type 2B vWD.

**Acquired von Willebrand Syndrome**

AvWS is similar to the congenital disease in terms of laboratory findings, being characterized by a prolonged BT and low plasma levels of FVIII–vWF [65]. About 270 cases of AvWS have been reported in the literature since the original description of a case associated with systemic lupus erythematosus [66].
The condition appears to be associated mainly with lympho-myeloproliferative disorders, immunological diseases and tumours. At variance with other acquired haemostatic defects (i.e. acquired FVIII deficiency), the presence of an inhibitor to vWF has been demonstrated in a relatively small number of cases. Characterization of the reported cases has consistently shown that vWF is normally synthesized but is rapidly removed from plasma through four possible pathogenic mechanisms [67]: (a) specific autoantibodies; (b) nonspecific antibodies that form circulating immunocomplexes and favour vWF clearance by Fc-bearing cells of the reticuloendothelial system; (c) absorption onto malignant cell clones; or (d) increased proteolytic degradation. The severity of the bleeding tendency varies from mild to life-threatening. The data of an International Registry of the clinical and laboratory parameters of patients with AvWS, organized on behalf of the Scientific Subcommittee on vWF of the International Society of Thrombosis and Haemostasis, have been published [68].

Genetic diagnosis and family studies
The aims of genetic diagnosis are to determine the causative defects of vWF when phenotypic diagnosis is certain, and make a definite diagnosis of vWD when it is uncertain. By definition, vWD results from mutations in the vWF gene. There are two main approaches to the genetic diagnosis of vWD: direct mutation detection and linkage analysis. At the moment, neither represents a first-line investigation for diagnosis. In the absence of a characterized mutation within a family, linkage analysis permits a defective vWF gene to be tracked, thereby corroborating phenotypic diagnosis. Screening for common genetic mutations can be obtained, mainly in type 2 vWD variants where clusters of mutations have been identified. In most type 1 and 3 vWD cases, direct mutation detection requires the sequence of the entire vWF gene. In these cases, gene tracking by analysis of restriction fragment length polymorphisms (RFLPs) or variable number of tandem repeats (VNTR) sequences of the vWF gene is useful for family studies. An on-line database of vWD mutations and polymorphisms is maintained by Ginsburg and colleagues and is accessible at http://mmg2.im.med.umich.edu [69,70]. Intron 40 of the vWF gene contains several VNTR sequences within a region of repetitive DNA. Analysis of these VNTRs is very informative and useful in gene-tracking studies in vWD [71]. These techniques have applications in the prenatal diagnosis of severe vWD, in carrier identification in recessive forms of vWD, and in type 1 vWD families when the diagnosis is phenotypically uncertain.

Prenatal diagnosis in vWD
The procedures available for prenatal diagnosis of vWD are identical to those developed and used for the prenatal diagnosis of haemophilia [72]. The preferred method is to obtain fetal tissue for DNA analysis by chorionic villus sampling after 10–12 weeks gestation. This procedure has become accepted in many centres worldwide as it can provide diagnosis in the first trimester. Alternative methods include amniocentesis or ultrasound-guided fetal blood sampling. Blood samples may be analysed for vWF:Ag as well for DNA markers. As most type 1 and 2 vWD are mildly affected, prenatal diagnosis is required mainly for the rare families with type 3 vWD in whom the threat of bleeding is similar to that of haemophilia A. In the few reported cases of prenatal diagnosis of vWD, the procedure was performed in a situation where a severely affected child already present within the family, so that the fetus was deemed to be at risk [71,73].

Management of patients with vWD
In patients with vWD the goal of therapy is to correct the dual defect of haemostasis, i.e. the abnormal platelet adhesion and abnormal intrinsic coagulation due to low FVIII levels. There are two treatments of choice in vWD, i.e. desmopressin and transfusional therapy with blood products. Other forms of treatment can be considered as adjunctive or alternative to these [1–3,74,75].

Desmopressin
Desmopressin (1-deamino-8-D-arginine vasopressin; DDAVP) is a synthetic analogue of vasopressin originally designed for the treatment of diabetes insipidus. DDAVP increases FVIII and vWF plasma concentrations without important side-effects when administered to healthy volunteers or patients with mild haemophilia and vWD [76]. The obvious advantages of DDAVP is that it is relatively inexpensive and carries no risk of transmitting blood-borne viruses. DDAVP is usually administered intravenously at a dose of 0.3 µg kg⁻¹ diluted in 50 mL saline, infused over 30 min. This treatment increases plasma FVIII–vWF 3–5 times above the basal levels within 30 min. In general, high FVIII–vWF concentrations last for 6–8 h. As the responses
in a given patient are consistent on different occasions, a test dose of DDAVP administered at the time of diagnosis helps to establish the individual response patterns. Infusions can be repeated every 12–24 h depending on the type and severity of the bleeding episode [77,78]. However, most patients treated repeatedly with DDAVP become less responsive to therapy [79]. The drug is also available in concentrated forms for subcutaneous and intranasal administration, which can be convenient for home treatment. The protocol of DDAVP test infusion with the clinical and laboratory parameters to be used to evaluate the biological response in each patient are summarized in Table 5; the definition of response to DDAVP is also reported together with the list of DDAVP products commercially available in Italy.

Side-effects of DDAVP are usually mild tachycardia, headache, flushing: these symptoms 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. A few cases have been described, mostly in young children who received closely repeated infusions [80]. Even though no thrombotic episodes have been reported in vWD patients treated with DDAVP, this drug should be used with caution in elderly patients with atherosclerotic disease, because a few cases of myocardial infarction and stroke have occurred in haemophiliacs and uraemic patients given DDAVP [81,82].

DDAVP is most effective in patients with type 1 vWD, especially those who have normal vWF in storage sites (type 1, platelet normal). In these patients FVIII, vWF and BT are usually corrected within 30 min and remain normal for 6–8 h. In other vWD subtypes, responsiveness to DDAVP is variable. A poor and short-lasting response is observed in type 1, platelet low [20]. In type 2A, FVIII levels are usually increased by DDAVP but BT is shortened in only a minority of cases. DDAVP is contraindicated in type 2B because of the transient appearance of thrombocytopenia [83]. However, there have been reports on the clinical usefulness of DDAVP in some 2B cases [84,85]. In type 2N, relatively high levels of FVIII are observed following DDAVP, but released FVIII circulates for a shorter time period in patient plasma because the stabilizing effect of vWF is impaired [86]. Patients with type 3 vWD are usually unresponsive to DDAVP.

Other nontrasfusional therapies for vWD

Two other types of nontransfusional therapies are used in the management of vWD, i.e. antifibrinolytic amino acids and oestrogens. Antifibrinolytic amino acids are synthetic drugs that interfere with the lysis of newly formed clots by saturating the binding sites on plasminogen, thereby preventing its attachment to fibrin and making plasminogen unavailable within the forming clot. Epsilon aminocaproic acid (50 mg kg\(^{-1}\) four times a day) and tranexamic acid (25 mg kg\(^{-1}\) three times a day) are the most frequently used antifibrinolytic amino acids. Both medications can be administered orally, intravenously or topically and are useful alone or as adjuncts in the management of oral cavity bleeding, epistaxis, gastrointestinal bleeding and menorrhagia. As drugs that inhibit the fibrinolytic system, they carry a potential risk of thrombosis in patients with an underlying prothrombotic state. They are also

| Table 5. Products containing Desmopressin commercially available in Italy. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Product (company) | Preparation | Volume per ampoule | Number of ampoules per pack | Comments |
| Emotin (Kedrion) | 4 µg | 0.5 mL | 10 | Concentrated ampoules can also be administered subcutaneously |
| | 20 µg | 1.0 mL | 10 | |
| | 40 µg | 1.0 mL | 10 | |
| Minirin DDAVP (Ferring/Valeas) | 4 µg | 1.0 mL | 10 | |

Infusion test with desmopressin (DDAVP)

Infusion protocol: administer over 30 min 0.3 µg kg\(^{-1}\) of DDAVP in 50 mL of saline. The same dosage can be administered also subcutaneously.

Clinical and laboratory parameters: factor VIII/vWF activities must be measured before and 0.5, 1, 2 and 4 h after the injection of DDAVP; bleeding time must be performed at least before and after 2 h.

Definition of responsiveness: vWD patients should be considered responsive to DDAVP when after 2 h they showed increases of baseline levels of FVIII:C and vWF:RCo of at least three-fold, with levels of at least 30 U dL\(^{-1}\) and bleeding time of 12 min or less.

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contraindicated in the management of urinary tract bleeding. Oestrogens increase plasma vWF levels, but the response is variable and unpredictable, so that they are not widely used for therapeutic purposes. It is common clinical experience that the continued use of oral contraceptives is very useful in reducing the severity of menorrhagia in women with vWD, even in those with type 3, despite the fact that FVIII–vWF levels are not modified.

Transfusional therapies

Transfusional therapy with blood products containing FVIII–vWF is at the moment the treatment of choice in patients unresponsive to DDAVP. Early studies indicated that cryoprecipitate administered every 12–24 h normalized plasma FVIII levels, shortened BT, and stopped or prevented clinical bleeding in vWD [87]. Based on these observations, cryoprecipitate has been the mainstay of vWD therapy for many years. However, a recent analysis of previously published reports has pointed out that BT is not always corrected following cryoprecipitate [88]. Virucidal methods cannot be applied to cryoprecipitate, therefore this product carries a small but definite risk of transmitting blood-borne infections. Therefore, virus-inactivated concentrates, originally developed for the treatment of haemophilia A, play an important role in the current management of vWD patients unresponsive to DDAVP. Concentrates obtained by immunoaffinity chromatography on monoclonal antibodies (FVIII > 2000 IU mg⁻¹) contain very small amounts of vWF and are therefore unsuitable for vWD management. Recently, a concentrate particularly rich in vWF and with a very low content of FVIII has also been produced [89]. This concentrate was clinically effective when tested in a small group of type 3 vWD cases [90]. The very low FVIII content in this product necessitates the infusion of purified FVIII concentrate to ensure haemostatic levels of this factor in the treatment of acute bleeding episodes and for emergency surgery. After 6 h, infused vWF triggers the endogenous synthesis of FVIII, so that no further infusion of FVIII-containing concentrates is necessary. Efficacy and safety of this concentrate are now under evaluation in a larger number of patients with different vWD types [91].

The dosages of concentrates recommended for the control of bleeding episodes are summarized in Table 6. The characteristics of products containing FVIII–vWF commercially available in Italy until December 2001 are summarized in Table 7. As commercially available intermediate and high-purity FVIII–vWF concentrates contain large amounts of FVIII and vWF, high postinfusion levels of these moieties are consistently obtained [92–95]. Furthermore, there is a sustained rise in FVIII, higher than predicted from the doses infused, lasting for up to 24 h. This pattern is due to the stabilizing effect of exogenous vWF on endogenous FVIII, which is synthesized at a normal rate in these patients [96]. The cumulation of exogenous FVIII infused with concentrates rich in this moiety, together with that endogenously synthesized and stabilized by infused vWF, is associated with very high FVIII levels when multiple infusions are given at frequent intervals for severe bleeding episodes or to cover major surgery. There is some concern that sustained high levels of FVIII may increase the risk of postoperative deep vein thrombosis [97].

FVIII–vWF concentrates are not always effective in correcting BT [98]. There are probably multiple reasons for the inconsistent effects on BT. So far, no concentrate contains a completely functional vWF, as tested in vitro by evaluating the multimeric pattern and using several functional assays, because vWF proteolysis occurs during purification due to the action of platelet and leucocyte proteases contaminating the plasma used for fractionation [99]. Despite their limited and inconsistent effect on BT, FVIII–vWF concentrates are successfully used for the treatment of vWD patients unresponsive to DDAVP, especially for soft-tissue and postoperative bleeding [52].

<table>
<thead>
<tr>
<th>Type of bleeding</th>
<th>Dose (IU kg⁻¹)</th>
<th>Number of infusions</th>
<th>Objective</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major surgery</td>
<td>50</td>
<td>Once daily or alternate daily</td>
<td>Maintain FVIII &gt; 50 IU dL⁻¹ until healing is complete</td>
</tr>
<tr>
<td>Minor surgery</td>
<td>30</td>
<td>Once daily or alternate daily</td>
<td>Maintain FVIII &gt; 30 IU dL⁻¹ until healing is complete</td>
</tr>
<tr>
<td>Dental extractions</td>
<td>20</td>
<td>Single</td>
<td>Maintain FVIII &gt; 30 IU dL⁻¹ for up to 6 h</td>
</tr>
<tr>
<td>Spontaneous or post-traumatic bleeding</td>
<td>20</td>
<td>Single</td>
<td>Maintain FVIII &gt; 30 IU dL⁻¹</td>
</tr>
</tbody>
</table>

FVIII, factor VIII.
persists despite replacement therapy, other therapeutic options are available. DDAVP, given after cryoprecipitate, further shortened or normalized BT in patients with type 3 vWD in whom cryoprecipitate failed to correct it [100]. Platelet concentrates (given before or after cryoprecipitate, at doses of 4–5 × 10¹¹ platelets) achieved similar effects in patients unresponsive to cryoprecipitate alone, both in terms of BT correction and bleeding control [101]. These data emphasize the important role of platelet vWF in establishing and maintaining primary haemostasis.

In summary, the therapeutic approaches to vWD patients according to vWD subtypes are reported in Table 8.

### Treatment of vWD during pregnancy and delivery

During pregnancy, vWF and FVIII levels tend to rise in type 1 and 2 vWD but this rise does not occur until the 10–11th weeks of gestation. No significant changes occur in patients with type 3 vWD. As improvements in vWF and FVIII levels during pregnancy are variable, patients should be monitored during pregnancy and for several weeks after delivery when levels fall rapidly and may cause late bleeding [60]. In type 1 vWD, FVIII levels are the best predictor of the risk of bleeding at delivery. The risk of bleeding is minimal when FVIII is > 50 U dL⁻¹ but can be significant when it is lower than 20 U dL⁻¹ [102]. Careful surgical haemostasis along with effective uterine contraction usually compensates for prolonged BT. In type 3 vWD, characterized by prolonged BT and low FVIII levels, replacement therapy with concentrates is necessary. Patients with type 2B vWD may develop or aggravate thrombocytopenia during pregnancy [40,41], but it is not clear whether thrombocytopenia exacerbates clinical bleeding.

### Treatment of patients with allo- and auto-antibodies to vWF

For the rare patients with type 3 vWD who develop anti-vWF alloantibodies after multiple transfusions, the infusion of vWF concentrates not only is ineffective, it may cause postinfusion anaphylaxis due to the formation of immune complexes [53–57]. These reactions may be life-threatening [56,57]. To overcome this reaction, a patient undergoing emergency abdominal surgery was treated with recombinant FVIII, because this product, being completely devoid of vWF, did not cause anaphylactic reactions. Due to the very short half-life of FVIII devoid of its vWF carrier, recombinant FVIII had to be administered by continuous intravenous infusion, at very large doses
sufficient to maintain FVIII levels above 50 U dL\(^{-1}\) for 10 days following surgery [57].

**Future directions**

Several aspects of the diagnosis and management of vWD must be tackled in the near future. Firstly, better characterization of type 1 vWD, the most frequent form of vWD is required. This can be achieved also with the contribution of molecular biology techniques, which can clarify the basic mechanisms of this type of vWD. Secondly, a more precise identification of vWD patients who are responsive to DDAVP. Thirdly, a better evaluation of the pharmacokinetics of FVIII:C following FVIII–vWF concentrate in vWD to establish whether or not there are indications for pure FVIII–vWF concentrates devoid of FVIII:C. Most of these questions will be answered when the results of several ongoing international studies become available in the next few years.

**References**

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