Nordic guidelines for diagnosis and management of von Willebrand disease (VWD)

**Guidelines of the Nordic Hemophilia Council**

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The guidelines have a planned validity until XXXX-XX-XX. A revision is performed biannually and the latest version can be found on the NHC website (http://nordhemophilia.org).

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### THE NORDIC HEMOPHILIA COUNCIL

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## *Diagnostic guidelines*

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### Background of von Willebrand factor and von Willebrand disease

#### Definition of von Willebrand factor and von Willebrand disease

* Von Willebrand Factor (VWF) is a large multimeric protein with two main functions in hemostasis. It is responsible for the flow-dependent tethering of platelets to the vascular injury sites, specifically adhesion to subendothelial collagen and bridging to other platelets via aggregation to secure platelet plug formation and primary hemostasis. Furthermore, VWF is a carrier protein for coagulation factor VIII (FVIII), which is thereby protected from degradation in plasma. VWF can also interact with fibrinogen and fibrin and can contribute to the clot formation process under certain situations (1).
* Von Willebrand disease (VWD) is a bleeding disorder caused by deficiency of and/or dysfunctional VWF. VWD is usually inherited (congenital), but rare acquired forms exist. Congenital VWD is divided into type 1, characterized by quantitative deficiency of VWF, type 2, by dysfunctional VWF disorder, and type 3, by lack of VWF. Type 2 is further subdivided into subtypes 2A, 2B, 2M and 2N, depending on the type of functional deficit in the VWF protein. Spontaneous or tissue injury-related mucocutaneous bleeding events are characteristic of VWD, due to the impaired primary hemostasis. These bleeds are due to non-optimal interaction between vessel wall and platelets, where the role of VWF is crucial.



**Figure 1.** Schematic illustration of the primary structure of the VWF monomeric polypeptide chain. The *VWF* gene is transcribed into an 8.8 kb long mRNA that is translated to 2813 amino acid precursor polypeptide, which consists of a 22 amino acid signal peptide (SP), a 741 amino acid long propeptide (domains D1 and D2) and a mature subunit of 2050 amino acids (D’-D3-A1-A2-A3-D4-B[1-3]-C[1-6]-C-knot). This propeptide and the terminal cysteine-rich knot (C-knot) are important for the initial dimerization of VWF in the endoplasmatic reticulum. In the trans-golgi network, the dimers undergo multimerization and proteolytic cleavages that yield mature multimers (not shown). The circulating VWF contain number of distinct functional domains, including binding to FVIII binding to platelet receptor glycoprotein (GP) 1b, binding to collagen, and binding to platelet receptor GPIIb/IIIa, the receptor for both VWF and fibrinogen. A cleavage site for ADAMTS13, which regulates the size of the VWF multimers, resides in the A2 domain.

#### Introduction to the biochemistry of VWF

* VWF is a circulating large glycoprotein with a concentration of approx. 10 mg/L in plasma. In healthy population, there is a 5-fold variability of VWF levels, and 25 % of activities of which is influenced by ABO blood group, 35% by genetics of VWF, and the remaining variability is explained by other genes (2 - 4) to cause rest of the variation. VWF protein is secreted into plasma from the EC by a continuous constitutive mechanism, whereas VWF from platelets is released only upon platelet activation. EC will release VWF from stores in the Weibel Palade bodies when exposed to various perturbation stimuli, including catecholamines, histamine and fibrin formation.
* The plasma form of VWF is a multimeric protein constructed of 2 - 40 dimer subunits of the protomer, resulting in a range of multimers with molecular weights ranging from 500 - 20.000 kDa. The mature VWF protomer hosts several well-characterized binding sites (Figure 1). Most importantly, regarding VWD, one binding site interacts with collagen and another site with GP Ib of the platelet surface contributing to platelet adhesion at the wound site. These particular functions of primary hemostasis depend on blood flow and the structure and molecular weight of VWF multimers, and subsets of low-molecular-weight multimers are regarded too small to provide a sufficient spacer function. The unfolding of the VWF protein is important, however, but this can be detected only under blood flow and is not captured by routine laboratory assays. In addition, the VWF subunit holds binding sites for FVIII, a RGD motif, recognizing the platelet GPIIb/IIIa during aggregation, and a site that interacts with heparins and heparin like -molecules. The specific site for FVIII in VWF provides a protective non-covalent binding, thereby limiting random proteolytic breakdown of FVIII. With our current understanding, VWF multimers are assembled in the Golgi apparatus of EC and if not directly exported, the multimers are retained in the Weibel Palade bodies. It has been suggested that ECs are also involved in the storage of FVIII.
* Following the release of VWF from ECs under blood flow, enzymatic exposure of VWF to a metalloprotease (ADAMTS13) reduces the largest VWF multimers in size. Not all cleavage sites will be exposed and the limited proteolysis results in a typical oligomer sub-band pattern (called triplet structure) that can be revealed by multimer analysis from plasma. The lack of VWF and mutations in the FVIII binding sequences of VWF may significantly reduce the plasma level of FVIII. In severe VWD, the FVIII values may be 2-10 % compared to healthy individuals.

#### Von Willebrand disease

*
* From aforementioned it is well understood that VWD is caused by a defect, inflicting platelet functions, and the major clinical hallmark in VWD is a tendency to mucocutaneous bleeds.
* The history of VWD dates back to Erik von Willebrand (5) who reported on a bleeding disorder, with fatal outcomes, denoted pseudohemophilia, occurring equally often in both sexes. Today, we know that VWD is a highly heterogeneous group of bleeding disorders with the common denominator of a quantitative or qualitative deficiency of VWF in circulating plasma, platelets, and endothelium. The basis for diagnosis of VWD relies on patient’s and relatives’ medical history, signifying an increased and objective bleeding tendency, together with a phenotype compatible with a defect of primary hemostasis.
* Since many variants of VWD are reported, the ISTH has developed a guideline for classification of VWD, simplifying the hierarchy of subclasses. Table 1 summarizes the current recommendation, including the amendments agreed upon by the ISTH in 2006 (6) and 2021 (7).
* In many variants of type 2 and 3 VWD, genetic defects have been identified, while in many individuals with type 1 the molecular defects remain unknown. Type 3 VWD is an autosomal recessive form with severe bleeding problems. The type 2 VWD expresses variable clinical manifestations but can be quite severe in some situations.
* The current classification recognizes four qualitative forms: 2A, 2B, 2M with a dominant inheritance pattern) and 2N that is a recessive disease. Thanks to large multicenter studies on type 1 VWD our knowledge of the complexities causing VWD have been extended (3, 7). Candidate mutations have been identified in approx. 65 % of the index cases with type I VWD whereas in the remaining cases the reduced VWF level may be caused by other factors, with the blood group AB0 gene showing the strongest effect. In later years, genetic data from genome-wide association studies have identified other genes that contribute to the plasma level variation of VWF (4, 8). VWF gene mutations are listed in an international web- based registry hosted by the EAHAD DBS organization (dbs.eahad.org).

**Table 1.** Types of von Willebrand disease

|  |  |
| --- | --- |
| **Type** | **Description** |
| 1 | Partial quantitative deficiency of VWF. There is one distinct subtype called type 1C, which is caused by markedly increased VWF clearance. |
| 2 | Dysfunctional (qualitative) VWF.  |
| 2A | Decreased VWF-dependent platelet adhesion with a selective deficiency of high molecular weight multimers. |
| 2B | Increased affinity for platelet GPIb. |
| 2M  | Decreased VWF-dependent platelet adhesion and/or decreased collagen-binding capacity without a selective deficiency of high molecular weight multimers. |
| 2N | Markedly decreased binding affinity for FVIII. |
| 3 | Virtually complete deficiency of VWF. |

#### Differential diagnosis

*
* Congenital or acquired vascular and connective tissue defects along with platelet function defects are to be considered in the differential diagnosis of the patient presenting with bleeding symptoms, especially if a history of mucocutaneous bleeding is present. Thrombocytopenia and platelet function defects, e.g., Bernard-Soulier syndrome, Glanzmann thrombasthenia, platelet-type VWD, secretion disorders, and acquired causes of dysfunction (e.g., drugs, uremia or hematological disorders) also impair primary hemostasis. In the surgical setting, the distinction must be made between a bleeding diathesis versus bleeding caused by insufficient surgical hemostasis (to be checked from the respective surgical files). A profuse generalized bleeding tendency is typical of coagulopathy, as for example encountered in association with disseminated intravascular coagulation (DIC). The term describes a complex of signs that increase the risk for type 2 diabetes, stroke, and coronary artery disease.

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### Diagnosis of VWD and its subtypes

The diagnosis of VWD is based on three main criteria:

1. the patient should have significant bleeding symptoms,
2. there should be a family history of appropriately diagnosed VWD or significant bleeding symptoms, except in recessively inherited subtypes, and
3. VWF levels should be significantly and repeatedly decreased.

As VWD is a heterogeneous disease, its classification by best standards requires a quite extensive laboratory armamentarium (see Table 2).

A clinician with special knowledge of coagulation disorders should diagnose VWD. General practitioners should refer the patient to a hematologist or a specialist working at the unit of coagulation disorders. The diagnosis should be done carefully in children due to the developing coagulation system and reassessed regularly, e.g. every 10 years, during lifetime, as ageing populations with bleeding disorders may be subject to overdoses of replacement therapy and, possibly, to the risk of thrombosis.

#### Initial hemostasis screening tests

* The activated partial thromboplastin time (APTT) and the Owren and/or Quick prothrombin time (PT) test are often normal, but the APTT may be prolonged in cases where FVIII level are below 30-40 IU/dL. The complete platelet count is usually normal with exception of a mild to moderate thrombocytopenia found in patients with VWD type 2B or the platelet-type VWD. The Platelet Function Analyzer (PFA)-100, and the upgraded version PFA-200, may be utilized as a primary hemostasis-screening test device to identify moderately or severely low VWF and some platelet function disorders. However, it has low sensitivity for mild defects in primary hemostasis and lacks specificity since it does not differentiate VWD from other platelet defects. Its strengths include whole blood environment and interaction with collagen under high-shear blood flow conditions. Irrespective if PFA is used or not, it is important that samples from patients with suspected VWD is sent to a specialized coagulation laboratory for further evaluation.

#### Specific tests for vwd diagnosis

* As shown by the algorithm in Fig. 2, the diagnosis of VWD is dependent on a significantly reduced platelet-dependent VWF activity (VWF:GIbR or VWF:GPIbM), with the exception of type 2N.
* The Nordic Hemophilia Council recommends that the diagnosis of VWD usually is made by repeated VWF activity levels below 35 IU/dL to confirm VWD diagnosis. However, the exact level can be flexible, and some individuals will be diagnosed having VWD with somewhat higher VWF activity levels, dependent on symptoms and family history after exclusion of platelet defects.
* In patients with a bleeding phenotype and mildly reduced VWF activity levels of 35 – 50 IU/dL, “low VWF” is preferred (after exclusion of platelet defects) rather than “VWD”.
* Measuring FVIII:C level is important as a prerequisite to approach the subtype 2N diagnosis. Additionally, FVIII plays an important role in assessment of the bleeding and likely thrombosis risks in VWD. However, in all subclasses of VWD the level of FVIII may be decreased, and it is particularly low (< 5 IU/dL) in type 3.
* The VWF antigen and activity tests, as well as tests for VWD subtyping, are described in Table 2 and discussed in more detail in the section below.
*

**Table 2.** Recommended laboratory assays used for diagnosis and subclassification in VWD.

|  |  |
| --- | --- |
| **Method** | **Diagnostic and monitoring purposes** |
| VWF antigen, VWF:Ag  | Quantitation of VWF antigen (protein). Used to differentiate between VWD type 1 and 3 or between type 1 and 2.  |
| Platelet-dependent VWF activitya) VWF:GPIbRb) VWF:GPIbM | The platelet-dependent VWF activity is the main functional method. The different assays have in common the capacity to capture VWF binding to the platelet receptor GPIb. The original assay, denoted VWF:RCo, utilizes reconstituted lyophilized platelets and ristocetin is not longer recommended to use. The VWF:RCo assay should be replaced by the VWF:GPIbR and VWF:GPIbM assays that utilizes recombinant GPIb fragments, with or without ristocetin, respectively. |
| VWF collagen binding, VWF:CB | Measures the capacity of VWF to bind to collagen. The assay is sensitive to detect multimer impairment. The discriminatory power is dependent on type of collagen used. Reduced VWF activity but normal multimeric pattern is indicative of VWD type 2M. |
| VWF multimer analysis, VWF:Multimers | Electrophoretic procedure essential for detection of multimeric size distribution (band pattern). The faster moving bands are denoted low-molecular weight multimers (LMWM) and the slower migrating bands are indicated as high-molecular weight multimers (HMWM). The protein bands in between are often called intermediate-molecular weight multimers (IMWM). The test can be performed with different gel concentrations that allows different resolutions of the bands. With high gel concentration it is possible to examine the oligomeric structure of the VWF bands (known as triplet) for further subclassification of VWD type 2 A variants (i.e., IIA, IIB, IIC, IID etc.). |
| FVIII binding capacity, VWF:FVIIIB | Determines the capacity of VWF to bind FVIII. Specific test for VWD type 2N. |
| Ristocetin induced platelet aggregation, VWF:RIPA | Determination of the ristocetin sensitivity using the patient’s platetet-rich plasma. Increased sensitivity (0.5 mg/mL or lower) is indicative of VWD type 2B. VWF:RIPA is absent in VWD type 3 and generally decreased in VWD type 2A. |
| VWF propeptide, VWFpp | The propeptide is released from VWF after synthesis. As it is synthesized in the same molar ratio of the mature subunit, but circulates in plasma independently from VWF, with different half-lives it is possible to estimate a VWFpp/VWF:Ag ratio. A reduced VWF:Ag and an increased VWFpp/VWF:Ag ratio are indicative of a VWD type 1 with increased clearance (e.g. VWD type 1C). |
| FVIII coagulation activity, FVIII:C | Determination of FVIII coagulation activity. A disproportionally reduced FVIII (compared to VWF) is typical for VWD type 2N. |

### Comments regarding laboratory methods

### *Platelet-binding activity*

* The clinical utility of traditional ristocetin cofactor (VWF:RCo) assay is compromised by the large assay variability, suboptimal sensitivity and high VWF detection limit. Alternative assays of VWF:RCo may overcome these disadvantages. Assays developed in recent years measure binding to GPIb, or fragments thereof, directly, or indirectly though specific antibodies and with or without ristocetin as modulating agent.
* Nomenclature for VWF activity assay methodologies has been established as follow: 1) assays based on fixed platelets and platelet agglutination in the presence of ristocetin has the old name VWF:RCo, 2) assays based on recombinant GPIb and ristocetin are termed VWF:GPIbR, and 3) assays based on the GPIb containing gain-of-function mutations are termed VWF:GPIbM (9, 10).
* The Nordic Hemophilia Council recommends the new VWF platelet-dependent VWF activity assays (VWF:GPIbR, VWF:GPIbM) over the VWF:RCo. Please note that the use of "VWF:RCo" is still widely used in scientific literature, and it is used in these guidelines, to describe VWF platelet-binding activity in general. Much of the data presented here is also based on results that were produced before the newer activity methods came into use. Thus, when VWF:RCo is stated in these guidelines, it refers to VWF platelet-binding activity without further specification of which activity assay was used.
* The VWF:GPIbR involves binding of active plasma VWF, in the presence of ristocetin, to a recombinant fragment of wild-type GPIb coated onto latex particles through a monoclonal antibody. The particles agglutinate with a decreased light transmission, which is directly proportional to the ristocetin-mediated plasma VWF:GPIb-binding activity (11). There is also a variant of this assay based on magnetic particles with chemiluminescent technology (12).
* The VWF:GPIbM utilizes a recombinant GPIbα fragment containing two gain-of-function mutations (G233V, M239V), which bind plasma VWF via the GPIb receptor in the absence of ristocetin and shear stress. Added latex particles coated with an antibody against GPIb will bind the VWF-recombinant GPIbα complex, inducing microparticle agglutination and decreased light transmission, which is directly proportional to the VWF:GPIb-binding activity in the plasma (13, 14). The gain-of-function mutations introduced into the GPIb fragments originate from the platelet type or pseudo VWD, which are characterized by spontaneous binding of VWF to platelets carrying the mutant GPIb, hence there is no requirement for ristocetin (15).

*Von Willebrand factor antigen (VWF:Ag)*

* The concentration of VWF in plasma is measured with immune assays. Common methods are based on the ELISA technique or using an automated latex-enhanced immunoturbidometric assays performed with coagulation analyzers. There is also an automated commercially available chemiluminiscent assay. The assay principles have similar diagnostic capacity, but the reproducibility is better using automated assays (16).
* Normal plasma concentrations of VWF:Ag may be found in patients having VWD due to qualitative defects in VWF. For example, most type 2N (if not compound heterozygous) and some patients with other type 2 variants may express quite normal quantities of antigen, which is, however, dysfunctional. Thus, a reduced ratio between VWF activity/VWF:Ag may indicate a VWD type 2 phenotype. A cut off <0.7 has been recommended for platelet-dependent VWF activity/VWF:Ag in order to suspect VWD type 2A, 2B or 2M (7). Similarly, a reduced ratio between FVIII:C/VWF:Ag may be used to identify patients with VWD type 2N.

*Von Willebrand factor collagen binding (VWF:CB)*

* The method relies on the ability of VWF to adhere to collagen and is usually performed as an ELISA, but automated assays are available (17, 18). The assay principle utilizes collagen as a natural ligand to VWF, immobilized either on the plastic surface of an ELISA-plate or on the surface of very small plastic and magnetic particles. After incubation with plasma and washing, the bound VWF is detected using an enzyme-conjugated anti-VWF antibody, and a specific substrate will provide a color or chemiluminescent signal that is proportional to the VWF collagen binding capacity. The source and type of collagen used in the assay may differ between different manufacturers, which also have different collagen formulations in their kits. In general, collagen type I or type III, or a combination of the two, is used. One commercially available reagent utilizes a synthetic triple-helical peptide of collagen type III (12, 18).
* The method reflects a biological function of VWF and contributes to the diagnosis of VWD by providing information about the quality of VWF. The VWF:CB and the platelet-dependent VWF:GPIbR or VWF:GPIbM activity assays often show positive correlation with the multimeric structure of VWF and are used to classify VWD type 2 variants. The type 2M subtype is identified as reduced VWF activity, usually the platelet-dependent activity, together with normal multimeric pattern. However, variants with selectively low VWF:CB (and normal platelet-dependent activity) have also been described (Favaloro et al., 2020). Thus, type 2M could be further divided into type 2MGPIb and type 2MCB, depending on the phenotype with the VWF activity assays. This also indicates that the VWF:CB assay should not replace the VWF:GPIbR or VWF:GPIbM assays but rather used as a supplementary assay. The approach to combine a platelet-dependent activity assay, and the VWF:CB assay has been shown to reduce diagnostic errors in clinical laboratories (12).

*Multimeric sizing electrophoresis techniques, VWF:Multimers*

* The assay evaluates the distribution of VWF molecules with different molecular weights (multimers). The study of VWF multimer composition in plasma is based on electrophoresis in a gel system suited for separation of macromolecules. Following separation, VWF molecules are electroeluted onto an immobilizing membrane where patterns of multimeric subsets are identified by means of an immuno-enzymatic or lumographic technique. The test is complex, cumbersome, and difficult to perform and interpret. Therefore, it should be performed only in specialized laboratories with long-term experience. However, in recent years, a CE-marked commercial standardized system with pre-casted gels has been available. The system is semi-automated, results are obtained within one day and it has been validated for VWD diagnostics (19 - 21).
* The method is most often qualitative (i.e., visual inspection of the multimer pattern) but quantification by integration of the area under a densitometric curve is possible which makes the interpretation of results easier. It is also possible to adapt the method by changing the gel system to focus on the large multimers or to increase the resolution in the low range, which may resolve abnormalities of triplet structure of each multimer (22). The commercially available assay, with pre-casted gels, is developed for multimer analysis at low resolution and therefore cannot be used to study aberrations of the triplet structure and other band abnormalities.
* In VWD type 1 all multimers are present, whereas VWD type 3 is characterized by loss of all multimers. VWD type 2A is often associated with a severe loss of large and intermediate multimers. Most type 2B patients display a loss of large multimers but exceptions, with normal multimers have been reported. Patients with VWD type 2M demonstrate all multimers, sometimes larger than normal (supranormal) or with bands resulting in a blurred (“smeary”) appearance. The multimers may also lack satellite bands in some type 2M samples. An aberrant multimer pattern may also be observed in type 2N due to abnormal disulphide bonds in the VWF molecule but the method cannot be diagnostically used in this subtype.

*Von Willebrand factor binding to FVIII (VWF:FVIIIB)*

* The VWF:FVIIIB is an important test for correct classification of VWD type 2N (Normandy), and it is able differentiate between type 2N VWD and hemophilia A. In principle, in the assay patient’s VWF is bound to an ELISA microtiter plate and incubated with highly purified FVIII. After extraction, the bound fraction of F VIII:C, if present, is then determined. An absent, or very low, FVIII binding activity is indicative for VWD type 2N. The commonly performed VWF:FVIIIB assays comprise validated homemade assays but recently a commercially and CE-marked assay has become available.
* The current ASH/ISTH/NHF/WFH guidelines suggest using either the VWF:FVIIIB or targeted genetic testing for patients with suspected VWD type 2N (7). The indication for the assay is when coagulation assessment reveals a low FVIII level in a patient with a negative family history of hemophilia. The low VWF may be due to a decreased carrier effect of VWF. VWD type 2 N is inherited as a recessive trait and patients are either homozygous or compound heterozygotes for different type 2N mutations. Usually, the type 2N patients have FVIII:C values <40 IU/dL, associated with normal or reduced VWF levels but a low FVIII:C/VWF:Ag ratio.

*Ristocetin-induced platelet aggregation (VWF:RIPA)*

* This test determines platelet aggregation as recorded in patient’s platelet-rich plasma (PRP) in the presence of ristocetin, using an aggregometer instrument. Aggregation occurs over a range of ristocetin concentrations and results from the ristocetin-induced interaction between VWF and platelet receptor GPIb complex. As the RIPA needs to be performed on fresh platelets it is necessary to perform the test within 2 h of blood collection.
* This method is relatively insensitive to quantitative deficiencies of VWF. The RIPA is absent in type 3 VWD and generally decreased in type 2A VWD. In type 1 VWD the RIPA will depend on the concentration of VWF in plasma, with a reduced RIPA at very low concentration of VWF.
* In contrast, type 2B variants display increased platelet aggregation to low concentrations of ristocetin. There is consensus that increased sensitivity to ristocetin concentration of 0.5 mg/ml or lower indicates the presence of type 2B VWD. Normal individuals will show in general platelet aggregation at and above 0.75 -1.0 mg/ml of ristocetin, but typically not below this concentration. In type 2B, this methodology is not well standardized, but usually ristocetin concentrations of 0.6 mg/ml or lower indicate a positive test result. Targeted genetic testing is recommended to confirm type 2B VWD diagnosis (see Genotyping VWD section).

*Von Willebrand factor propeptide (VWFpp)*

* The VWF propeptide separates from the VWF precursor after VWF is secreted to the circulation. The propeptide is a large (100 kDa) glycopeptide and can be measured immunologically using the ELISA technique with a specific antibody.
* The propeptide is important for correct dimerization and multimerization of VWF that will be secreted. However, it has no known function in the circulation. The VWFpp method represents a tool for identifying patients with acquired von Willebrand syndrome but also to characterize VWD types with shortened VWF half-life in plasma (increased clearance) (23 - 25). In such cases, the ratio of VWFpp/VWF:Ag is high, as VWF, unlike the propeptide, is cleared rapidly. Thus, the VWFpp can be useful to differentiate between severe VWD type 1, i.e., type 1C, from type 3 and to distinguish specific VWD type 1 variants (25). Furthermore, identifying increased clearance of VWF is of value when considering desmopressin treatment.

*Anti-VWF antibodies*

* Alloantibody formation against VWF is rare, but may be detected in patients with VWD type 3 that have been treated with VWF-containing concentrates (26). Autoantibodies to VWF can sometimes be associated with acquired von Willebrand syndrome.
* The presence of allo- or autoantibodies that neutralize the VWF activity can be tested and quantified based on the same principle as anti-FVIII antibodies (i.e., Bethesda assay). In brief, the patient plasma is mixed with pooled normal plasma and then the VWF activity is measured and compared with a control of pooled normal plasma mixed with VWF-deficient plasma. As the antibodies may be directed against different parts of the VWF molecule, mixing experiments should include different activity tests, i.e., which examine the ability to bind GPIb and collagen. However, detecting neutralizing antibodies in patients who have normal/subnormal levels of VWF activity is difficult with the conventional Bethesda assay. A variant of the assay that better considers endogenous VWF activity in the sample was described by Mannucci et al., (27) which means that the patient plasma is mixed with normal plasma while two control samples are analysed, where the two control samples together give the "expected" value in the sample. Antibodies can be also detected by a so-called ELISA-based method, which involves immunological detection of anti-VWF antibodies. The anti-VWF ELISA detects all types of antibodies, both neutralizing and non-neutralizing antibodies.

*DDAVP challenge (biological response)*

* To ensure sufficient response for clinical use most patients should be administered a DDAVP test dose. Testing children is usually omitted until the age of 4 years. Depending on the mode of administration and suitability for the patient the recommended test dose is 0.3 µg/kg intravenously or subcutaneously, and, even intranasally, This testing profile is designed to assess the response to DDAVP by measuring VWF and FVIII levels pre-treatment (baseline) and at two time-points (1 and 4-hours) post-treatment. Post-treatment VWF and FVIII levels are evaluated against baseline levels to ensure the increase and sustained levels. DDAVP is usually effective in patients with type 1 VWD whose baseline VWF and FVIII levels are higher than 10 U/dL. A good response to DDAVP is defined as an increase of peak VWF and FVIII activity levels at least 3-fold over the baseline levels. A further blood sample collected after 4 hours is advisable to exclude patients having a short half-life of released VWF and/or FVIII following DDAVP stimulation. Type 1C (C for clearance) is characterized by a significant decrease (<30 %) of the 4 hours post-infusion from the peak VWF levels (28).



**Figure 2.** Recommendations of a complete laboratory test repertoire and algorithm for investigating suspected VWD and for subtyping into VWD type 1, 2A, 2B, 2M, 2N and 3. The threshold given the VWF activity/VWF:Ag ratio is not absolute and must be interpreted in the context with the patient’s clinical history and other laboratory findings. Ratios based on very low levels, close to the assay detection limit, should not be used, as the higher imprecision of the assays in the low measuring range may increase diagnostic errors. Patients with low VWF activity levels (35-50 IU/dL) and significant bleeds should be recognized as a distinct group, named VWF low. In VWD type 2A and type 2B a concomitant decrease of both platelet-dependent activity and collagen-binding activities are often observed. In VWD type 2M reduced platelet-dependent VWF activity, or both platelet-dependent and collagen-dependent activities (together with normal VWF multimers) are typical, but variants with selectively low platelet-dependent activity or collagen-dependent activity can be seen, denoted VWD type 2MGPIb and type 2MCB, respectively. An increased RIPA refers to VWD type 2B, which usually coexists with an increased platelet aggregation at low ristocetin concentrations of 0.6 mg/mL or lower. All type 2 variants can be confirmed by genetic analysis, and especially recommended in type 2A/2B.

**Practical diagnostic considerations**

*Pre-analytical considerations*

* Clear preanalytical patient guidance regarding infection, time after potential surgery, and smoking, fasting or heavy exertion (should not be less than 24 hours) should be provided.

***Recommendations:***

* **Blood samples should preferably be collected in the coagulation laboratory to avoid preanalytical challenges.**
* **Samples should ideally be collected from fasting and resting subjects who have refrained from smoking and caffeine ingestion on the day of testing. Exercise, stress, infections, and pregnancy all elevate VWF and FVIII levels and may obscure the diagnosis of mild VWD type 1. Blood can be collected without consideration of the menstrual cycle (29, 30).**
* **Use a standardized, atraumatic blood collection protocol with minimal stasis.**
* **In children and teenagers, application of anesthetic plaster prior to blood sampling should be used.**
* **Use needles between 19 and 21 gauge to prevent vein trauma or reduced blood flow, leading to platelet activation.**
* **The first 3-5 mL of blood should not be used for VWD testing.**
* **VWD tests require the use of 105-109 mmol/L (3.2 %) buffered trisodium citrate tubes for blood collection.**
* **Tubes must be filled completely to ensure the proper 9:1 ratio of blood to anticoagulant.**
* **The collection tubes should be mixed gently immediately after filling, avoiding excessive agitation or mixing.**
* **Care should be taken to remove platelets from plasma by centrifuging to prevent platelet VWF from contaminating plasma on freezing and thawing the sample. Some laboratories use double centrifugation. If not tested immediately, plasma samples should be frozen immediately at -70° C**
* **If transportation of plasma samples is needed, samples should be kept frozen. If not frozen there is a risk of cold activation if cooling of the plasma sample occurs during transportation. This results in decreased VWF and FVIII activities and, therefore, may lead to misdiagnosis of VWD (31).**
* **Screening tests should include complete blood count with differential leukocyte analysis and platelet count, Owren and/or Quick PT, APTT, VWF activity (we recommend to use both platelet-dependent and collagen binding activity assays), VWF:Ag, FVIII level, and ABO blood type. In urgent situations, the use of PFA-100/200 may help to exclude moderate and severe forms of VWF. Prolongation of the closure times is sensitive but not specific for VWD and may indicate anemia, thrombocytopenia, platelet dysfunction or antiplatelet agents.**
* **Defining the subtype of VWD: A ratio of VWF:activity/VWF:Ag <0.7 defines type 2 VWD and RIPA and VWF:CB and/or VWF:multimers, and VWF:FVIIIB further defines the type 2 subtype. Mutational analysis may also help to define the VWD subtype. The use of VWF:CB and VWF:multimer assays will reduce potential errors in VWD misidentification and may add information in the differentiation between 2A and 2M subtypes (32, 33).**

*Genotyping VWD*

* Genotyping of VWD has its main impact when the VWD phenotype is difficult to discern, and it helps to find the differential diagnosis, such as hemophilia A or platelet defects. Genetic analysis is also useful in prenatal diagnostics and in genetic counseling. With new and powerful genotyping techniques, the knowledge about new variants and modifying genetic factors is steadily increasing. However, pathogenic variants are not always easy to be identified in all VWD subtypes, especially in VWD with mild clinical phenotype (i.e., VWD type 1). Pathogenic variants are most often found in severe quantitative VWF deficiency (VWD type 3) and in qualitative deficiencies (VWD type 2), in contrast, this is not the case in <65% of VWD type 1 alleles (34 - 36).
* VWD type 1 is the most prevalent subtype, and it is in majority of cases dominantly inherited but some patients may display recessive inheritance with more than one mutation. The mutations are located throughout the VWF gene. Genetic analysis in case of suspicion of VWD type 1 is not recommended for other purposes than exclusion of VWD.
* The locations of the VWD type 2 mutations depend on the VWD subtype, 2A, 2B, 2 M or 2N, but are usually found in a limited number of exons. Most often, they are caused by missense mutations that change specific amino acids, resulting in either loss- or gain-of-function phenotypes. In type 2A, the mutations are most often caused by variants in exon 28 that encodes the A2 domain of the VWF molecule or by mutations in exons 22 and 25-27 (D3 domain). Variants causing type 2A have also been found in exons 11-16 (D2 domain) and in exons 51-52 (CK domain). In type 2B, the pathogenic mutations are almost invariably gain-of function variants found in exon 28, in the region encoding the A1 domain, leading to enhanced interaction with platelet GPIb molecule, and leaving the in vivo hemostasis deficient. In VWD type 2M the mutations are found in exon 28 (A1 domain) or exons 29-32 (A3 domain). The inheritance of VWD type 2 N is recessive and the mutations, found in exons 17-20 or 24-25, impair the binding of FVIII to VWF and result in shortened FVIII half-life and thus, lower levels of FVIII in plasma.
* VWD type 3 is inherent in a recessive way and the pathogenic variants are also found throughout the entire VWF gene, but null mutations, resulting in complete loss of VWF synthesis, are found in majority of described cases. An investigation from 2013 concluded that VWD type 3 in Finland is caused mainly by two founder mutations, unlike reports from other populations (37).
* Mutations causing VWD and polymorphisms of the VWF gene can be found in an online VWF database on Internet (dbs.eahad.org) that is supported by the European Association for Haemophilia and Allied Disorders (EAHAD).

**Bleeding symptoms**

* VWD is characterized by prolonged and reoccurring mucocutanous bleeds, e.g., epistaxis and menorrhagia, bleeding after tooth extraction or surgery, and bleeding after minor wounds. Typically, the bleeding symptoms originate from several sites. Joint bleeds and frequent gastrointestinal bleeds occur in the most severe cases (38). Suggestive mucocutaneous bleeding symptoms are defined as:
* Nose bleeding, ≥2 episodes without a history of trauma not stopped by short compression of <10 min, or ≥1 episode requiring blood transfusion.
* Cutaneous hemorrhage and bruising with minimal or no apparent trauma, as a presenting symptom or requiring medical treatment.
* Prolonged bleeding from trivial wounds, lasting ≥15 min or recurring spontaneously during the 7 days after wounding.
* Oral cavity bleeding that requires medical attention, such as gingival bleeding, or bleeding with tooth eruption or bites to lips and tongue.
* Spontaneous gastrointestinal bleeding requiring medical attention or resulting in acute or chronic anemia, unexplained by ulceration or portal hypertension.
* Heavy, prolonged, or recurrent bleeding after tooth extraction or other oral surgery, such as tonsillectomy and adenoidectomy, requiring medical attention.
* Menorrhagia resulting in acute or chronic anemia, or requiring medical treatment, not associated with known structural lesions of the uterus, e.g. congenital defects, myomas.
* Bleeding from other skin or mucous membrane surfaces requiring medical treatment (e.g., eye, ear, respiratory tract, genitourinary tract, other than uterus).
* Newborns can show cephalohematoma, umbilical bleeding or bleeding after circumcision.

**Bleeding score**

* A bleeding score (BS) has been developed in a large European cohort of patients with type 1 VWD with an aim to quantitatively evaluate the severity of bleeding symptoms and correlation with clinical and laboratory features (39). The BS showed a strong significant inverse relation with VWF:RCo, VWF:Ag or FVIII:C. Higher BS was related with increasing likelihood of VWD, and a mucocutaneous BS was strongly associated with bleeding after surgery or tooth extraction. The relative importance of different bleeding symptoms was also described (Fig. 3) (39). ISTH has developed a standardized bleeding questionnaire and a defined interpretation grid for computation of a final BS, also referred to as the ISTH/SSC Bleeding Assessment Tool (BAT) (38). This kind of BS is a useful screening tool for VWD, and its use is encouraged at the Nordic Hemophilia Centers, see appendix 1. A version, better suited for pediatric patients, was published in 2009 (40).



**Figure 3.** Symptoms strongly suggestive of VWD show variability, but bleeds from several sites refer to a generalized hemostasis problem occurring often in VWD . Association between bleeding symptoms and type 1 VWD in enrolled families in an age-adjusted logistic model is shown. Index cases are excluded from the analysis. The graph reports the logistic estimate and its 95 % confidence interval (from Tosetto et al.(39)). Incidence of postpartum hemorrhage varies, and usually is associated with low FVIII levels, short half life of VWF and severe subtypes (41)

**Criteria for family history**

* A positive family history compatible with VWD (except for types 2N and 3) usually is associated at least with one first-degree relative, or two second-degree relatives, who have a personal history of objective, significant mucocutaneous bleeding events and laboratory tests compatible with VWD. When available, the use of VWF mutations or genetic markers linked to the VWF locus may allow examination of more distant relatives and may allow asymptomatic relatives with low VWF levels to provide evidence of inheritance.

**Clinical criteria for VWD type 1**

* Type 1 VWD: is a hereditary bleeding disorder due to quantitative deficiency of VWF. In most cases type 1 is inherited as an autosomal dominant trait. The diagnosis therefore is based upon criteria for symptoms, VWF deficiency, and inheritance, all of which must be satisfied. These include significant mucocutaneous bleeding tendency, laboratory tests with VWF levels below 35 IU/dL at least in two occasions and either a positive family history for VWD type 1 or an appropriate VWF mutation. Asymptomatic individuals are typically children who have not yet been challenged with trauma or invasive procedures that could cause bleeding.

*Special considerations on the diagnosis of type 1 VWD*

* In the investigation of type 1 VWD, the bleeding history is particularly important. The relative impact of bleeding manifestations observed in the European study (MCMDC-VWD1) is presented in Fig. 3, giving the odds ratio of various bleeding symptoms for the risk of a VWD based on 154 families studied (39).
* However, the biochemical diagnosis of type 1 VWD in persons with a mild deficiency in VWF represents a diagnostic dilemma. In three major cohorts, genetic analysis failed to detect mutations in approximately 35 % of patients with a type 1 VWD diagnosis (42). Clinically, patients with a very low concentration of circulating VWF most often present with a distinct bleeding tendency, hereby qualifying for a bleeding disorder diagnosis, while other patients with less suppressed and marginally low levels of VWF and equivalence between VWF activity and VWF:Ag constitute a grey zone between a healthy state and overt VWD. Linkage studies have further revealed that linkage between members in VWD type 1 families and the VWF gene locus is weak, when VWF:RCo levels are >45 IU/dL9 (34). Recent work has further demonstrated an inverse relationship between VWF mutations and levels of VWF in these patients. “Genetically confirmed” VWD type 1 has a high diagnostic sensitivity only at VWF:Ag and VWF:RCo levels below 35 IU/dL (Fig 3), which seems a reasonable diagnostic cut-off level. However, it should be noted that marginally low VWF levels not qualifying for VWD criteria, may be associated with increased mucocutaneous bleeds as shown in two case-control studies (30, 43) and that such individuals may carry VWD associated mutations (44).
* Low VWF: subjects with borderline VWF levels of 35-50 IU/dL may carry a risk for bleeding events rather than a disease. However, these individuals may be completely asymptomatic, unless a coinciding hemostatic risk factor would appear. Patients with borderline VWF levels and bleeding problems should be referred to a hematologist with knowledge of coagulation disorders. The distinction between low VWF trait and definite VWD type 1 will be useful for certain clinical and genetic studies. Alternative or additional diagnoses should be re-considered for patients with possible VWD, especially including the co-existence of platelet disorders.
* The presence of blood group O generally predicts lower levels of VWF compared with the non-O state and blood group O itself represents a risk factor for lowered VWF and may show increased bleeding tendency. The influence of the blood group on circulating VWF is not caused by differences in expression rates but is rather due to a blood group-dependent shift in clearance.
* Based on recent progress in understanding of type 1 VWD, it is advisable not to assign a diagnosis of VWD type 1 to persons with intermediately lowered plasma VWF (i.e. 35 – 50 IU/dL), but rather to denote symptomatic persons with VWF in that range as having a mild bleeding disorder or a risk for bleeding under situations which compromise hemostasis. This tendency and VWF levels may improve with aging.



**Figure 4.** Schematic model that illustrates factors influencing the VWF levels in plasma and the relationship with the hereditability. Dominant negative mutations refer to mutations that are dominantly inherited and have negative effects on the protein function and/or integrity (modified from ref. no. 45).

**Criteria for VWD type 2**

* VWD type 2 is defined by low levels of functional VWF activity (platelet-dependent activity and/or collagen binding activity), and a low VWF activity /VWF:Ag ratio (<0.7) (except type 2N). Type 2 is further subdivided into subtypes 2A, 2B, 2M and 2N, depending on the type of functional defect (Table 2, Fig 2).
* Type 2A and the classical form of type 2B lack the high molecular weight multimers, whereas the rare subtype of 2B called the type Malmö/New York has all multimers. Also, type 2M have a full set of multimers, albeit the separate bands may be aberrant. RIPA is decreased in type 2A and 2M but increased in type 2B.
* Type 2A is inherited as a dominant trait, but a recessive inheritance has been described (46). Two groups of mutations in the A2 domain of the VWF subunit cause the lack of HMWM in type 2A (21). Group 1 mutations affect intracellular transport, assembly, storage, and secretion of VWF multimers, and group 2 mutations cause increased susceptibility to proteolysis in plasma (47). Type 2B is caused by mutations in the A1 domain and is characterized by an increased sensitivity to ristocetin in the RIPA test. In addition, mutations in the D1 and D2 domain of the propeptide or in the D3 domain of the mature protein may cause a multimerization defect by affecting intramolecular disulfide bonding within the D3 domain (48, 49).
* Type 2M is similar to type 2A and characterized by a decreased binding of the VWF to platelets, but in contrast to 2A, all multimers are present. Mutations have been found in the A1 domain of the VWF. The type 2M-Vicenza subtype is characterized by the presence of multimers that are larger than normal and mutations are found in the D3 domain (50).
* FVIII deficiency is the typical feature of type 2N VWD due to FVIII binding defect caused by specific mutations in the VWF gene. The levels of FVIII ranges from 1 – 40 IU/dL but is usually above 5 IU/dL (51). The phenotypic diagnosis of type 2N is based on measuring the ability of VWF to bind FVIII (VWF:FVIIIB assay). The FVIII/VWF ratio is typically decreased (<0.7), but it may be only slightly decreased in compound heterozygotes for a type 2N mutation and a mutation causing a quantitative VWF deficiency. Very low VWF:FVIII binding capacity (<20 %) is indicative of VWD type 2N in homozygous or compound heterozygous state.

Criteria for VWD type 3

* VWD type 3 is inherited as a recessive trait and is defined by virtual absence of VWF and very low levels of FVIII (<10 IU/dL). It is very rare, with a prevalence of about 2-3 cases per million in the Nordic area. Higher figures have been diagnosed in countries with a high degree of consanguinity. Bleeding symptoms are usually moderate to severe, and many type 3 patients require replacement therapy with VWF-containing concentrates. The patients are usually homozygotes or compound heterozygotes for mutations in the VWF gene. Missense mutations are found in approx. 15 – 20%, which can result in a less severe phenotype compared to patients having 2 null alleles (3). Bleeding symptoms are more prevalent in obligatory carriers than in the normal population, but not as frequent as in patients with type 1 VWD (52).

**Deviations from the 2021 ASH/ISTH/NHF/WFH guidelines**

* Our main deviation from the ISTH/ASH guidelines is the limit (below 35 IU/dL) of VWF activity for a definite VWD diagnosis. Mild bleeding symptoms are common in normal life, blood group O and some undiagnosed platelet defects or other acquired disorders (e.g. endocrinological diseases), and are thus often identified by the general practitioner, particularly in association with mucosal bleeding from dental procedures, epistaxis, or menorrhagia. The comprehensive questionnaire to distinguish normal from abnormal bleeding, together with the use of an appropriate bleeding assessment tool, is critical. Diagnosis of VWD, based on a higher limit of the VWF levels, can lead to increased patient morbidity and health care system burden as well as unnecessary intervention delay due to additionally confirmatory steps for patients who require urgent or elective surgery and can lead to unnecessary medical anxiety. Thus, it may hamper the use of antithrombotic medication when indicated (53). A higher limit for VWF may also preclude another relevant diagnosis, such as enhanced fibrinolytic potential or platelet function disorder. Nevertheless, the clinical phenotype together with the laboratory phenotype establishes new VWD diagnoses, with the emphasis on the clinical phenotype.
* A second deviation is our recommendation to use both VWF platelet-dependent and collagen-dependent activities. The reason for this is to avoid missing VWD type 2M variants that may have a selective reduced activity of only one of the activities i.e. type 2MGPIb and type 2MCB, respectively (33).
* A third alteration refers to the assessment of clinical phenotype (BAT) by a clinician with special expertise on coagulation disorders. The personnel of primary care are not trained enough to perform BAT and they should refer the patients to a hematologist or a specialist working at the unit of coagulation disorders to order the relevant laboratory tests and management accordingly.

**Acquired von Willebrand syndrome**

* Acquired von Willebrand syndrome (AVWS) is a rare disorder associated with low levels of VWF and FVIII, but in comparison with inherited VWD, bleeding symptoms appear later in life without a family history. The majority of cases are caused by lympho- or myeloproliferative disorders. Other causes include solid tumors, immunological disorders, increased shear stress, hypothyroidism, drugs and miscellaneous or unknown causes (54, 55). The pathophysiology is not always clear. In hypothyroidism, the synthesis of VWF is decreased but in most other conditions, synthesis seems to be normal, but clearance is increased through different possible mechanisms: specific autoantibodies, non-specific autoantibodies forming immune complexes, adsorption to malignant cell clones or increased proteolytic degradation. In contrast to acquired hemophilia, inhibiting antibodies can only rarely be demonstrated.
* It may be difficult to distinguish AVWS from congenital VWD and the symptoms are similar, i.e. mostly mucocutaneous bleeds. However, usually there is an abrupt change in the bleeding pattern. Laboratory findings may vary between different causes of AVWS but it usually include mildly decreased VWF:Ag and FVIII:C and more pronounced decrease of the VWF activity assays. The VWF activity/VWF:Ag ratio is therefore frequently decreased. The large multimers are often lacking due to increased clearance. Thus, these patients often exhibit a laboratory phenotype that resembles VWD type 2A. However, it is important to note that patients with AVWS may have VWF antigen and activity levels above the cut-off for VWD. Inhibitors against VWF may be detected with Bethesda-like mixing studies and it is important to test for neutralization with both VWF activity assays (platelet-dependent and collagen binding) as only one of the activities may be affected. Non-neutralizing antibodies may cause an increased clearance of VWF without inhibiting the functional VWF assays and these antibodies are detected with an appropriate ELISA assay. Measurement of the VWFpp levels may also be useful as the VWFpp/VWF:Ag ratio is increased in plasma to a greater extent than most VWD types (except VWD type 1C), but the assay is not generally available. Since 2004, ISTH has run an international register of AVWS, and today there are just over 200 patients included. There is also an ongoing initiative by NHC to collect retrospective data from patients diagnosed with AVWS in the Nordic countries. We encourage the participation in this type of data collection in order to increase our knowledge in this complex area.

**Guidelines on treatment and management of VWD**

**Introduction**

* In VWD, bleeding tendency is caused by decreased levels or by inappropriate function of VWF, and sometimes in addition low levels of coagulation FVIII. The deficiencies can in general be corrected either by stimulating the release of endogenous VWF and FVIII with desmopressin (DDAVP) in VWD type 1, or by substitution with a VWF/FVIII concentrate in type 2 and 3. DDAVP may temporarily normalize hemostasis if functional levels of VWF and FVIII can be reached by endogenous release. VWF/FVIII or purified VWF concentrates are to be used when DDAVP is not an alternative.
* Plasma-derived concentrates carry a potential risk of transmission of infectious agents, but the risk has been negligible with the current safety actions, including specific protein purification steps combined with pathogen inactivation procedures. Recombinant VWF has been produced but is not yet available in all the Nordic countries. Cost is an issue, as these concentrates are expensive. On the other hand, safety may be compromised with DDAVP due to its modest or short-acting effects, side effects, mainly caused by the strong antidiuretic mechanism that may limit its use. Antifibrinolytic treatment (tranexamic acid) is an important adjuvant to DDAVP or concentrates, or as a single hemostatic agent, especially in connection with mucous membrane bleeds. Oral contraceptive pills and progesterone releasing IUD may be used as treatment of menorrhagia in females with VWD, as they typically alleviate menstrual blood loss.
* VWD is a complex condition to manage, and it is important that the patient is treated at a designated European Hemophilia center with access to a multidisciplinary team of specialists. The European association for haemophilia and allied disorders (EAHAD) is a European association of hemophilia and allied disorders, operating since 2007. EUHANET is an EAHAD-affiliated network that provides uniform recommendations for management of hemophilia and allied disorders (https://www.euhanet.org/). It has defined the services to be provided by comprehensive care centers (EHCCC) and hemophilia treatment centers (EHC). A multidisciplinary team should take care of the clinical decision making, typically in association with anesthesia, surgery, pregnancy, and delivery. Interaction between the clinicians and the specific laboratory needs to be available for 24/7 in the EHCCC. Also, a safety surveillance register of EUHASS is prospectively running to capture all side effects and complications, including mortality among patients with type 1 severe (<15 IU/dL VWF:RiCo), type 2, type 3 or type unknown.

Hemostatic agents

The different hemostatic agents used for treatment of bleeding and/or handling different clinical procedures in VWD patients include the following drugs:

* Desmopressin (DDAVP)
* VWF concentrates
* Tranexamic acid
* Oral contraceptive pills and IUD

**Choice of treatment**

The choice of treatment depends on several factors:

* Nature of the bleed or invasive procedure.
* Subtype and severity of VWD - level of functional VWF and FVIII.
* Previous bleeding history and response to treatment.
* Duration of treatment - single doses or a long-term treatment.
* Outcome of the DDAVP test - post DDAVP level and half-life of functional VWF and FVIII.Age of the patient. Restricted use of DDAVP is advised in frail elderly and the youngest children of less than 2 years of age - due to increased risk symptomatic hyponatremia in and an increased risk of thrombotic complications in patients with cardiovascular risk factors, including high age.
* The presence of other diseases that may contraindicate use of a therapeutic agent.
* Pregnancy and delivery.

Suggested treatment options are given in figure 5, in the text below and in appendix 1.



**Figure 5.** Management algorithm. Tranexamic acid should be given to all patients unless there is a contraindication. Repetitive doses of tranexamic acid will load the tissue and are often useful for hemostasis by inhibiting early fibrinolysis. A single dose of tranexamic acid can be used in case there is a high risk of thrombosis during an acute bleed or major surgery. DDAVP is generally inefficient or contraindicated in patients with type 1C VWD in the setting of surgery, and in type 2B and type 3 VWD patients. Desmopressin may be used in some instances of mild bleeding for type 2 VWD, mainly some 2M variants with confirmed responses in the DDAVP trial. The choice between VWF replacement therapies (FVIII/VWF 1:1, 1:2, 0:1) is made based on local practices and the clinical assessment of thrombosis risk. OD: on demand; DDAVP: desamino-8-arginine vasopressin.

**Desmopressin**

* Desmopressin (1-desamino-8-D-arginine vasopressin, DDAVP) is a synthetic analogue of vasopressin, initially used for the treatment of diabetes insipidus. Desmopressin was designed to have prolonged duration without hemodynamic effects. In the mid-1970s it was first reported that desmopressin at high dosage stimulated the release of endogenous FVIII, VWF and tissue plasminogen activator (t-PA). The effect is immediate, with on average 2-6-fold increases in plasma concentrations of FVIII, VWF and t-PA. Optimal hemostatic effect is achieved with a dosage of 0.3 µg/kg given intravenously. A higher dose will not improve the response (56). Subcutaneous or intranasal spray administrations are both effective and suitable for home treatment. The response to subcutaneous or intranasal administration is of comparable magnitude, but somewhat slower in onset than that of intravenous administration.

*Dose and modes of administration*

* 0.3 µg/kg i.v. or s.c.
* 300 µg i.n. (spray) (150 µg if BW<30/50 kg)

Intravenously (i.v.): slow injection of DDAVP (diluted in saline to 10 mL) during 15 minutes or infusion (diluted in 50 – 100 mL saline) during 30 minutes diluted in 50-100 mL saline. Peak FVIII/VWF levels are observed at 60 minutes.

Subcutaneously (s.c.): Peak FVIII/VWF levels are reached after about 120 minutes.

Octostim® solution (15 µg/mL) is the most suitable for s.c. administration, due to its high concentration. Often a single 15 µg dose s.c. will suffice in adults.

Intranasal (i.n.) spray: Peak FVIII/VWF levels are reached at 120 minutes.

One spray to each nostril will provide the normal adult dose of 300 µg. For patients with a body weight <30 kg, a dose of 150 µg is recommended (one spray in one nostril). In small children (body weight less than about 15 kg) the spray should not be used.

*Contraindications*

* Hyponatremia, cystic fibrosis, poorly controlled hypertension, acute coronary syndrome, tendency for severe migraines/headache and severe renal disease. Smokers are at risk for thrombosis in association with DDAVP administration, and DDAVP is at least a relative contraindication. In children weighing below 15 kg body weight and in pregnancy, DDAVP is not recommended and should be used carefully.

*Dosage intervals*

* Twelve to 24 hours is the ordinary dose interval. The risk of severe hyponatremia must be noted if repeated doses are given. The patient should be put on fluid restriction if repeated doses are given, the limitations of fluid intake is 1.5 liter per day of administration. Tachyphylaxis may develop after repeated dosing.

**Treatment with desmopressin related to the nature of the bleeding episode**

*Major bleeds*

*Response criteria:*

* DDAVP can be used for treatment of bleeds in patients in whom the administration leads to normal or at least >50 IU/dl VWF:RCo and FVIII:C levels. The levels should increase at least 2-3 fold and remain at least above 50 IU/dL 4 hours post DDAVP (consensus opinion). In connection with life threatening and other severe bleeds, a VWF/FVIII concentrate should be administered as the first line treatment. If the response to DDAVP is suboptimal or the duration is short, a VWF/FVIII concentrate should be administered.

*Minor bleeds*

*Response criteria:*

* VWF:RCo and FVIII:C should reach a level of at least 30 IU/dL within 2 hours after DDAVP and last for over 4 hours.

*Type of invasive procedure*

* All procedures – VWF and FVIII activities should reach normal levels within the first 2 hours and stay elevated ≥ 30 IU/dL for at least 12 hours post DDAVP.
* If treatment ≥ 3 days is required, tachyphylaxis and antidiuretic effects may lower the efficacy. Sodium and factor levels should be monitored, and managed accordingly, with low VWF levels replacement therapy should be commenced.
* Treatment varies with the procedure and more options at different procedures are given in appendix 2.
* Desmopressin may still be useful in some instances of mild bleeding for type 2 VWD, mainly some 2M variants with confirmed responses in the DDAVP trial.

**Limitations with desmopressin**

* Consider the subtype of VWD, duration of treatment and age of the patient.
* Half-life is short in type 1C VWD, consider against treating with desmopressin in the absence of a desmopressin test (4 h).
* Patients with classical type 2B VWD should not be given DDAVP due to subsequent platelet aggregation and thrombocytopenia. Patients with VWD type 3 are non-responders to DDAVP. Patients with type 1C VWD do not benefit from DDAVP in the setting of surgery
* Half-life of FVIII:C may be short in 2N.
* DDAVP should preferably not be given to small children (<15 kg body weight, elective testing 4 years), and adult patients with cardiovascular disease or comorbidities (e.g., history of angina pectoris, myocardial infarction, stroke, peripheral arterial occlusive disease, arrhythmias, and epilepsy).
* If DDAVP is administered to young children, fluid administration must be restricted, and electrolytes monitored closely. Upon repeated dosing to patients of any age, fluid administration should be restricted.
* Duration of treatment should normally not exceed 3 days. Treatment may be prolonged if factor levels and sodium are monitored, but tachyphylaxis has been reported after several doses of treatment with DDAVP.
* Precaution in pregnancy due to possible side effects for both the pregnant women and the fetus/child.

**Adverse effects with desmopressin**

* The adverse effects of DDAVP include tiredness, headache, nausea, decreased appetite, temporary lowering of blood pressure with secondary tachycardia, facial flushing, fluid retention, hyponatremia and seizures, which will limit its use.

**Tranexamic acid**

* Tranexamic acid is an antifibrinolytic agent. It interferes with the fibrinolysis of newly formed clots by binding to the lysine-binding sites of plasminogen thus inhibiting its binding to fibrin. Administration can be oral, intravenous, or topical (e.g., as mouthwash). It can be used alone (e.g., in the management of epistaxis and menorrhagia) or in combination with DDAVP or VWF concentrates. To increase its effectiveness, tranexamic acid should be given prior to elective procedures and with repetitive dosing to ensure concentrations in tissues as well.

*Available products in the Nordic countries*

* Tranexamic acid solution for injection (100 mg/mL) and tablets of 500 mg. In Sweden, and on special permission in Denmark, dissolvable tablets (1g) are available. In Sweden, also oral solution with 100mg/ml is available, especially for children.

*Dose and modes of administration*

* Orally 20-25 mg per kg BW 3- 4 times daily for 7-10 days.
* Intravenously 10 mg per kg BW 3-4 times daily for 7-10 days.
* Mouthwash 10 mL of a 5% solution 4 times daily, which can be swallowed.

**Limitations with tranexamic acid**

* Contraindicated in the management of upper urinary tract bleeds.
* Dose reduction is necessary in patients with renal insufficiency.
* Should be avoided, or its usage minimized, in patients with a recent thromboembolism and/or a previous personal thromboembolic disease and/or strong risk factors for thrombosis.
* No data are available on the use of tranexamic acid in newborns.

**Adverse effects with tranexamic acid**

* The adverse effects include nausea, vomiting, diarrhea, and abdominal pain.

**VWF concentrates**

*Important properties*

* Several properties are to be considered when choosing a concentrate for treatment of VWD. Adequate virus inactivation is a prerequisite. The VWF may be somewhat functionally inactivated during the manufacturing process, and in vivo, which may be reflected by a low ratio between VWF activity and antigen, or by an abnormal multimeric pattern. The ratio between VWF activity and FVIII:C is important to consider when dosing the concentrate. VWF activity in relation to amount of total protein (specific activity) gives information about purity. The use of pure VWF concentrate provides the way to control/maintain the natural FVIII levels in prophylactic use.

*The multimeric structure of the VWF*

* The VWF is a multimeric protein, the largest multimers (HMWM) probably being the most effective for binding platelets during the formation of platelet plug, i.e., in primary hemostasis. The functional and clinical importance of different multimeric sizes is, however, still not fully understood.
* An aberrant multimeric structure may indicate that the VWF is dysfunctional due to proteolysis during manufacture or afterwards. The multimeric structure may be visualized with electrophoretic methods and objectified by densitometry. An indirect method is to calculate the ratio between the VWF activity (most often measured as VWF:RCo and VWF:Ag.). A low VWF:RCo/VWF:Ag ratio indicates loss of functional activity. Concentrates with a VWF:RCo/VWF:Ag ratio > 0.7 are probably to be preferred.

*Ratio between VWF activity and FVIII:C*

* Most concentrates used for treatment of VWD contain both VWF and FVIII (except Willfact/Wilfactin, which is characterized by a high VWF:RCo/FVIII:C ratio and Veyvondi, a recombinant human von Willebrand factor product). A concentrate with a high relative amount of FVIII (a low VWF:RCo/FVIII:C ratio) may increase plasma levels of FVIII above normal, as the infused FVIII adds to the patient’s endogenously released FVIII. Even if patients with VWD may have very low basal levels of FVIII in plasma, they do have the ability to produce and release FVIII, if VWF becomes available in plasma. Therefore, the infused VWF will stimulate synthesis and release of FVIII. When repeated doses are given, FVIII levels should be monitored, and factor doses adjusted to avoid high FVIII levels (above 150-190 IU/dL). Also, the relation between the half-lives of VWF:RCo and FVIII:C should be considered. A concentrate with a short half-life of VWF:RCo in relation to FVIII:C may impose an increased risk of high FVIII levels, if it has to be dosed frequently.

**Dosage of VWF concentrates**

* Concentrates used for VWD should be dosed according to the VWF:RCo content, which therefore must be labeled on the vials. The recovery of VWF:RCo in adults is roughly 1.5 - 2.0 IU/dL per infused IU VWF:RCo/kg body weight. A dose of 50 IU/kg can be expected to increase the VWF:RCo with about 75 - 100 IU/dL. Therefore, a loading dose of 50-60 IU VWF:RCo/kg body weight is recommended for patients with very low basal levels of VWF:RCo.
* In general the half-life of VWF:RCo is considered to be equal to that of FVIII:C. Therefore, VWF concentrate is administered every 12-24 hours in association with surgery and similar conditions. VWF concentrate can also be given as a continuous infusion.
* When used for prophylaxis in outpatients, a VWF concentrate administered 2-3 times per week is usually sufficient to prevent bleeds.
* Levels of VWF:RCo and FVIII:C should be monitored when repeated daily doses are given over a longer period. Measurement of the VWF:Ag level is not sufficient as the VWF may become dysfunctional (57).

*Concentrates for VWD approved in the Nordic countries:*

* Haemate®, CSL Behring A plasma-derived concentrate with a VWF:RCo/FVIII:C ratio of about 2.
* Wilate®, Octapharma A plasma-derived concentrate with a VWF:RCo/FVIII:C ratio of about 1.
* Wilfactin® Willfact® /Willefact® LFB Currently available in Denmark, Norway, and Finland. A plasma-derived concentrate with a VWF:RCo/FVIII:C ratio of approx. 60. This is to be considered to avoid exogenous additive FVIII, if the patient and/or the invasive procedure have strong thrombogenic properties. When managing a bleed, the first dose may be supported by one dose of FVIII infusion.
* Veyvondi® (Vonvendi®), Takeda, is the first recombinant high-molecular weight VWF concentrate without FVIII. When managing a bleed, the first dose may be supported by one dose of FVIII infusion. Currently available in Sweden and Norway and Denmark.

**Management of specified bleeds or invasive procedures**

*Bleeds from nose and mouth*

* Bleeds from nose and mouth are relatively common especially in younger patients with VWD. Tranexamic acid given orally (mixture or tablets) or locally is often sufficient to stop these bleeds. In case of oral bleeds, mouthwash with tranexamic acid (the i.v. solution or a chewed tablet) may be effective.
* If tranexamic acid is not sufficient to control the bleeds, DDAVP or a VWF-containing concentrate is indicated.
* Prolonged or recurrent nasal bleeds may require local treatment, e.g., nasal cautery or laser, and treatment with tranexamic acid over a longer period. Regular local treatment with Vaseline or similar may reduce the bleeding tendency from nosebleeds.

*Dental extractions*

* In mild cases, minor dental extractions with local anesthesia may be carried out under the cover of tranexamic acid only. The treatment could start the day before or at least 4 hrs prior to the extraction and can be repeated three times daily for several days. In more severe VWD cases, or in connection with extensive procedures, and if regional anesthesia, or an inferior dental block is given, DDAVP or a VWF-containing concentrate should be added to tranexamic acid. A single dose of DDAVP or VWF-concentrate is often sufficient. Tranexamic acid should be continued for about 5-7 days both as mouthwash and per orally. The respective dosage levels are specified above. All local procedures fostering primary hemostasis, such as fibrin glue, are recommended.

*Menorrhagia – heavy menstrual bleed*

* Treatment options for menorrhagia in females with VWD include tranexamic acid, DDAVP, VWF concentrates, oral combined contraceptive pills, and intra-uterine progesterone contraceptives. Tranexamic acid reduces menstrual blood loss with about 50%. Tranexamic acid is to be taken only during the menstrual period, in some cases only during the first days of menstruation. If tranexamic acid and oral contraceptives are not sufficient, DDAVP or a VWF concentrate is needed to control the menstrual bleed. DDAVP and concentrates are typically needed only during the menstruation days. DDAVP is usually restricted to three consecutive days because of the risk of fluid retention. NSAIDs should be avoided, but selective Cox-2 inhibitors (coxibs) may be of value as pain medication.

*Gastrointestinal bleeds*

* Gastrointestinal (GI) bleeding is the most frequent cause of hospitalization in VWD. Patients often present with recurrent overt or occult GI bleeding from an unidentified source and clearcut anemia. This clinical picture is assumed to be related to an increased incidence of GI angiodysplasia in VWD.
* The mechanism is associated with dysregulated angiogenesis related to the lack of VWF high molecular weight multimers since GI bleeding from angiodysplasia is most frequent in VWD types 2A, 2B, and 3 and in acquired von Willebrand syndrome.
* Diagnostics: The management of angiodysplasia has been improved with the advent of video capsule endoscopy (VCE), which is now the gold standard investigation to detect small-bowel angiodysplasia. If bleeding source is not identified with conventional endoscopy, VCE is the tool to identify lesions eligible to argon plasma coagulation (APC), the most effective endoscopic therapy that can be performed using the double balloon endoscopy.
* Despite a high prevalence of angiodysplasia in VWD, specific guidelines are not available for the modalities of GI tract exploration in patients with GI bleeding.
* Treatment: Argon plasma coagulation (APC).
* Angiogenesis inhibitors have successfully been used in some case reports.

**Red blood cells and iron deficiency**

* Management of anemia, usually due to iron deficiency is critical in VWD. VWF mediates platelet adhesion to vascular injury sites under high shear rates, exceeding 800 1/s. The shear forces are dependent on blood flow rates and hematocrit, which assists VWF to reach the mechano-resistance for the platelets at the hemostatic site (58). Red blood cells have also other roles in hemostasis, as they bind to fibrin, provide procoagulant surface platform and transform to polyhedrocytes sealing the wound site (59). Overall, anemia is impairing the physiological VWF functions, and should be avoided. Typically, repeated bleeding episodes will lead to iron deficiency anemia (IDA). It should be managed, at an elective situation by peroral iron substitution. In case of poor absorbance and response and emergency situations or repetitive bleeding episodes, intravenous iron administration is the method of choice for the treatment target (60).

**Surgery and other invasive procedures**

* Surgery and other invasive procedures should be performed in close association with or at a center with clinical and laboratory expertise in bleeding disorders including VWD, and with a coagulation laboratory that can measure VWF and FVIII activities around-the-clock. Both FVIII and VWF levels must be evaluated, depending on the type of procedure. Active VWF is needed to cease mucous membrane bleeds, and VWF should be normalized in connection with invasive procedures involving mucous membranes. FVIII activity is an important determinant for surgical and soft tissue bleeds and therapeutic levels should be reached during and after surgery for a period of 3-10 days (depending on the type of procedure). FVIII:C and VWF activity (VWF:RCo or VWF:CB) should be monitored in association with all major surgical procedures. During surgery and the first post-operative day, normal levels of FVIII:C and VWF:RCo levels should be reached. Anemia and thrombocytopenia need attention. With a prolonged bleeding, especially from GI tract, FXIII levels may be consumed, and it is worthwhile to monitor them.
* Repeated infusions of a VWF/FVIII concentrate may induce unnecessarily high levels of FVIII:C in plasma, due to the additive effect of the endogenously released FVIII. Very high FVIII:C levels (>150 IU/dL) or VWF:RCo (>200 IU/dL) over a longer period postoperatively should be avoided, because of the risk of thromboembolic complications. A concentrate devoid of FVIII is suitable for patients having risk of thrombosis. Thromboprophylaxis is not traditionally used, but may become an issue in obese and cancer patients.

*DDAVP and surgery*

* DDAVP can be used in responsive patients (prior laboratory and clinical efficacy testing), but the risk of water retention and tachyphylaxis must be considered if repeated doses are given. This may limit the usefulness of DDAVP. In some cases, a combination of DDAVP and a VWF concentrate may be useful. DDAVP may be administered via intranasal spray before minor procedures, but in connection with major procedures, it is advisable to give DDAVP parenteral either i.v. or s.c.
* DDAVP intranasal spray in a dose of 300 µg i.n. (150 µg if BW <30 kg) should be administered about 60 minutes before the invasive procedure. Intravenous (i.v.) or subcutaneous (s.c.) DDAVP should be given at a dose of 0.3 µg /kg about 30 (i.v.) or 60 minutes (s.c.), respectively, before the procedure. Fluid and electrolyte balance should be monitored when prolonged treatment is given.

*VWF/FVIII concentrate for surgery and invasive procedures*

* Patients who do not respond to DDAVP should be given an approved concentrate containing VWF. In case of major surgery, in patients with severe VWD, treatment should be given for at least 1-2 weeks post surgery. As the recovery of VWF:RCo is about 1.5 – 2.0 IU/dL per infused IU VWF:RCo/kg BW, a loading dose of 50-60 IU VWF:RCo/kg i.v. is recommended for patients with very low basal levels of VWF:RCo. The ensuing doses can usually be lower, about 25-40 IU VWF:RCo/kg i.v. every 12-24 hours. After 24-48 hours a once daily dose, or a dose every other day, may be sufficient for the first post-operative week. FVIII:C should be monitored to avoid high levels over a longer period of time. FVIII and VWF activity levels of ≥0.50 IU/dL for at least 3 days after major surgery is usually recommended. NB! Purely VWF containing product should be started on the preoperative day or together with FVIII replacement just before the intervention.

*Tranexamic acid and surgery*

* Tranexamic acid should be given in addition to DDAVP or VWF concentrate, especially if the procedure involves mucous membranes.
* Tranexamic acid is administered at a dose of 10 mg/kg i.v. about 30 minutes before surgery or 20-25 mg/kg orally about 2 h before surgery. Thereafter the tranexamic doses are repeated with 6-8 h intervals for at least a week postoperatively.

*Platelet transfusions*

* Platelet concentrates should be considered if treatment with VWF concentrate fails to control a bleed in patients with severe VWD. The patient’s platelets are also devoid of functional VWF and therefore donor platelets may be of help at the local site of hemostasis.

*Thromboprophylaxis*

* Thromboprophylaxis should not be given routinely to patients with VWD undergoing surgery. Low molecular weight heparin in a prophylactic dose may be considered in patients with multiple or severe prothrombotic risk factors in association with high doses of a VWF/FVIII concentrate. Furthermore, VWF activity and FVIII:C should be >50 IU/dL if thromboprophylaxis is given. Compression stockings are recommended in association with major surgery.

**Women and VWD**

* All women’s aspects related to VWD need to be evaluated, diagnosed, and managed together with hematologists and gynecologists, and pediatric doctors in case of planning a delivery (Figure 6). Women/girls represent majority of population with VWD (61%), as reported by a 1092 patient-study from the Netherlands, and accordingly women/girls are referred to special centers more often than men/boys (61). The mean age of the first bleed, was 8.9 years in boys and 10.6 in girls. The time delay from the first bleed to the diagnosis was 7.7 years for boys but 11.6 years for girls, illustrating the need for improvement.



**Figure 6.** VWF around the circle of life of women. From conceiving, early childhood to adulthood there are typical spontaneous and risk situations influenced by VWD. Miscarriages, abnormal organogenesis and angiogenesis are a possibility. The general hemostatic risks and impairments caused by hypertension, anemia, hypocalcemia/magnesemia and endocrinological aspects should be managed appropriately throughout life. Comorbidities will need special attention, and multidisciplinary approach. C-section, cesarian section, GI, gastrointestinal, IUD, intrauterine device, PPH, postpartum hemorrhage.

**Menorrhagia and its management**

* In women, menorrhagia is the most typical symptom of bleeding disorders affecting primary hemostasis. Platelet disorders and VWD are the most frequent underlying conditions. Almost all women with VWD (89-99%) suffer from menorrhagia (62, 63). It is typical that menstruation is heavy and lasts for several days. This usually leads to iron deficiency anemia (IDA), which further enhances the bleeding tendency. Thus, prevention and management of iron deficiency is critical to improve hemostasis and overall quality of life of women with VWD. The same applies to repetitive gastrointestinal bleeds.
* Despite this knowledge, the self-reported outcome studies imply that 40% of VWD patients do not receive attention to heavy menstrual bleeds (HMB). Accordingly, almost half of the patients had also suffered from the often-neglected IDA (64). After the laboratory diagnosis of iron deficiency confirms the etiology of anemia, oral or intravenous iron should be commenced. If there is an urgent need to improve erythropoiesis and/or oral iron is not tolerated or insufficient, iron should be administered intravenously.
* Combined hormonal contraception with estrogen or other hormonal treatment (such as intrauterine device, IUD with progesterone) offers a suitable means to simultaneously manage heavy menstrual bleeds and contraception.
* Menstrual blood loss is diminished with estrogen containing oral contraceptive pills and progesterone IUD in women with VWD, even in type 3 patients. Estrogens increase the plasma level of VWF, except in patients with type 3 VWD. However, the response is variable and unpredictable. The mechanism of action is partly dependent on the increased level of VWF and partly on the local effect on the endometrium. Gonadotropin-releasing hormone may be of further help, if recommended by the gynecologist.
* Massive uterine bleeds need to be managed with interventional radiologists, or angiologists, who may use embolization techniques to stop the bleeds.
* Either DDAVP (patients with VWD type 1 and some patients with VWD type 2 according to a desmopressin test and other functional tests) or VWF replacement therapy is indicated temporarily on the days of heavy bleeds, unless VWF is not in prophylactic use. Tranexamic acid is useful for all types of VWD, if tolerated. Tranexamic acid at repeated doses loads to the tissues and is having a prolonged pharmacodynamic action together with the other treatment modalities.
* Menstrual pain has been advised to be controlled with non-steroidal anti-inflammatory drugs. However, COX2-inhibitors, coxibs, are best for the hemostatic purpose since the prostaglandin and arachidonic acid metabolites are directed towards some platelet activation, rather than their inhibition.

**Menopausal bleeds**

* Fibroid formation and menopause can cause problematic bleeds not only in general, but especially if the primary hemostasis is compromised. This needs to be discussed with the patient and the gynecologist in advance to have a management plan in place. Again, the role of managing anemia and hormonal regimens as well as local interventional management tools, such as hysterectomy or thermal balloon endometrial abrasion, are important

**Cancer and cancer surgery**

* The patients with VWD should be treated for cancer in the same way as the patients without bleeding diathesis, but the specifics of hemostasis need to be delineated. In case of invasive procedures or surgery, the need for factor concentrate or desmopressin in combination with tranexamic acid should be evaluated and a written plan and laboratory follow-up are to be provided. Since some types of chemotherapy can cause thrombocytopenia, the bleeding tendency could increase in patients with VWD. With good clinical planning together with the oncologist, anesthesiologist and surgeon regarding management of cancer and hematological treatment, the hemostatic routines can be established.
* Some types of cancer, for instance myeloproliferative disorders, multiple myeloma, and sometimes solid tumors can be associated with acquired von Willebrand syndrome. It is therefore important to check blood samples on a regular basis, and on demand upon increased bleeding symptoms.
* Since women with VWD often have menorrhagia, bleeding symptoms due to a gynecologic cancer, may be overlooked and the diagnosis may be delayed. It is therefore important to have regular appointments with gynecologists, and acutely in case of changing bleeding patterns.
* Cancer patients may become thrombogenic, and therefore high VWF/FVIII levels (above 150 IU/dL) should be avoided in association with replacement therapy and surgical settings. Pure VWF concentrates are often preferred under these circumstances. In some extreme cases with high thrombotic burden, thromboprophylaxis with low-molecular weight heparin can be considered. Some types of cancer, for instance myeloproliferative disorders, multiple myeloma, and sometimes solid tumors can be associated with acquired von Willebrand syndrome. It is therefore important to check blood samples on a regular basis, and on demand upon increased bleeding symptoms.

**Abortion and miscarriage**

**Pregnancy**

* Deliberate abortion needs replacement support aiming at factor (VWF and FVIII) above 50% and tranexamic acid to control fibrinolysis. Oral hormonal contraception or local hormonal intrauterine device (IUD) is recommended as alternatives if pregnancy is not planned near-term.
* Insemination and early organogenesis may be influenced by VWD, but the literature is conflicting regarding patient outcome. Increased miscarriages rates have been reported by a patient-reported outcome study (65), but not in a single center pilot study from Canada although the numbers of miscarriages were 25% in 20/80 pregnancies in VWD patients versus 16% in 50 control pregnancies (66) (p-value 0.28). A case-control study did not either show increased risk of placental abruption, preterm delivery, fetal growth restriction or stillbirth (67). Later larger studies have supported these data, both in type 1 or type 3 VWD (68).
* In case of pregnancy, a written follow-up plan on the laboratory assays, their timing, replacement therapy, interventions and concomitant medications is useful and needed to secure the safety of the patient and the next steps, also covering the emergency hours.
* Deliveries should be managed by a team, which is familiar to the plans, and the pediatric doctor should be involved as early as possible when a child with VWD or a bleeding disorder of unknown origin will be or is born.
* Follow-up during pregnancy is important, but there is no consensus on how often this should be done. In some centers, both clinical and laboratory testing is carried out in each trimester, while other centers only perform a planned examination in the last trimester. A clinical and laboratory examination is necessary if an increased bleeding tendency is noted. It is important to follow the complete blood count, including platelet counts, which sometimes decrease related to pregnancy itself. Thrombocytopenia should be noted in association with VWD and especially with type 2B, and low platelet count may bring new challenges to the management. Moreover, it can be valuable to screen the coagulation factors, including fibrinogen, D-dimer (disproportionate fibrinolysis) and FXIII in selected cases. If the pregnant woman in addition to VWD has thrombotic propensity, thrombophilia, family history or significant obesity or fatty liver, the balancing with hemostatic and possible anticoagulation are relevant.
* If bleeding episodes occur, this needs interim attention including the clinical and laboratory assessment and possibly VWF prophylaxis, if the VWF values do not rise or are rapidly eliminated, which typically occurs along pregnancy in severe VWD type 1 and in some forms of type 2. However, in some cases of type 1 the half-life of VWF is low and should be observed by evaluating the trough values at 4-6 hours after VWF administration.
* Another issue is normal cationic calcium and magnesium levels since they are hemostatic relevant players. Calcium is needed in all steps of hemostasis and platelet functions. Especially, magnesium is involved in FIX activation and platelet-collagen interactions and should be in balance and under normal references with calcium (69, 70).
* Mode of delivery should follow the obstetrical decision-making together with the pregnant woman and the hematologist. Vaginal delivery is to be preferred, and the indications for caesarean section are mainly obstetrical, considering both the mother and the baby.

**Delivery**

* It is important to have a written plan in patient files regarding the approaching delivery, latest at the week 36. The management of pain relief is an essential part of the preparations.

**Epidural/spinal anesthesia (neuraxial anesthesia)**

* It is difficult to evaluate the hemostatic thresholds in VWD for neuraxial anesthesia, since the evidence is based on small, retrospective studies and case reports (71). As a general rule, women with VWD type 1 can have epidural/spinal anesthesia if their VWF activity and FVIII level are more than 50 IU/dL, e.g., within normal reference values. VWF activity levels should be maintained at least at 50 IU/dL while the epidural is in place and for at least 6 hours after its removal. For type 2 and type 3 VWD an individual assessment is necessary, depending on the actual factor levels of VWF activity, FVIII, and red cell and platelet count and prophylactic VWF administration plans and their responses. The risk for procedure-associated bleeding needs to be related to other risks, for instance with general anesthesia.

*Replacement therapy*

* On individual basis, as VWF and FVIII tend to rise physiologically during pregnancy, the VWF dose and type of concentrate should be evaluated. Note that a pure VWF replacement therapy would not add to endogenous FVIII levels unless given at least 6 hours ahead of the delivery. A pure VWF concentrate is a valuable alternative to manage the patient, especially if thrombotic risk factors are present. Women with type 3 VWD typically do not show any increase of FVIII and VWF during pregnancies because endothelial VWF stores are lacking. Thus, VWF/FVIII concentrates are required to manage delivery to keep the levels above 40 IU/dL for vaginal delivery and above 50 IU/dL for cesarian section (72). Replacement therapy should be prolonged up to 5–7 days to maintain FVIII and VWF activity levels > 50 U/dL, but usually not above 150-190 IU/dl.

**Postpartum hemorrhages (PPH)**

* The days after delivery, the VWF and FVIII:C activities are falling whereby bleeding may be an issue. Postpartum hemorrhage may also occur due to uterine injury, its retention or extra-uterine tissue injuries. In addition, wound healing is impaired in VWD, since not only plasma, but also tissue and platelet VWF is reduced. Hemostatic support is therefore necessary according to laboratory analysis at each obstetric unit. A preparatory plan should be in place for a case of PPH (73). Rapid bedside estimation of coagulation or needs for transfusion by thrombelastometry (ROTEM or TEG) is of value, however, it does not detect the contribution of VWF, whereby actual plasma samples and traditional laboratory analysis are needed. Iron deficiency anemia should be recognized and well managed not only antepartum, but also postpartum, and especially after PPH.

**Pediatric aspects**

**Newborn babies**

* If the father or the mother has VWD, the risk for inheriting VWD in the newborn is 50% in most cases. Newborns with potential VWD can be born by vaginal delivery but instrumental delivery by forceps or vacuum extraction should be avoided. For severe cases of VWD with a VWD activity below 20% in the family, also, intramuscular injections and scalp electrodes, should be avoided and cord blood can be used to facilitate a quick diagnose. However, in most cases of suspected VWD, testing for VWD should be postponed since VW-activity in newborns can show doubled values compared to adult reference values (44-235%), misleading the diagnosis. For children without bleeding symptoms and family members with VW-activity above 20%, testing for VWD is recommended at the age of around one year. If the testing is done earlier, it is recommended to be repeated. Children with VW-activity above 20% can receive intramuscular injections, i.e., vitamin K or vaccinations.

**Other pediatric issues**

*Diagnostics*

* In children, the bleeding score ISTH-BAT is recommended and can be used to capture bleedings (74, 75). A pediatric version is available with additional questions on neonatal bleedings. However, bleeding scores in children should be evaluated with caution since children often have not experienced any significant bleeding challenge. Therefore, despite a possible bleeding disease, the score can be low. For diagnostic subtyping of VWD, if possible, an adult family member should undergo the complete VWD diagnostics to minimize extensive blood draws, especially in RIPA analysis. Results of VWF levels should be interpreted with caution in children, especially if the blood draw was traumatic with a high stress level. When interpreting results, age-related values should be taken into account. However, general reference intervals for children, especially for infants, are missing for many coagulation assays. It is possible to find information about expected values obtained from healthy children in various pediatric text books and published studies, but the results should be interpreted with caution by laboratory expertise against the background of the local conditions regarding methods and other aspects.

*Medication*

* For all medication, it is important to adapt the medication regularly due to growth and weight gain. Parents should be informed to avoid NSAID (Ibuprofen, Naproxen) for fever episodes in children with VWD and instead use Paracetamol. For inflammation or pain, also, celecoxib has been used. Regarding tranexamic acid, in some Nordic Countries, beside tablets, tranexamic acid is also available as oral solution and soluble tablets, which is useful in preschool children and can also be used locally, e.g. nose and mouth bleeds. DDAVP should not be used in children below 15 kg body weight. If DDAVP is administered to young children, fluid administration must be restricted, and electrolytes monitored closely. Beside i.v. - and s.c.-injections, which are dosed by body weight, also nasal spray is available for older children from about 5 years of age.
* For teenage girls with heavy menstruations, oral contraceptives could be discussed or alternatively, small intrauterine contraceptive device suitable for that age group can be a solution, see chapter on women and VWD.
* For children with severe VWD type 3, helmet and knee protection in the first years of life could be of value. A written emergency plan to daycare and school with information of disease and how to act in the case of bleeding is recommended for all children diagnosed with VWD.
* As already mentioned in the pregnancy section, the hemophilia center should have a written plan for the pregnant mother as well as for the newborn. This must address all necessary steps for the newborn regarding diagnosis and treatment in case of bleeding etc.

**Management of outpatients**

* Patients with VWD should regularly visit a hemophilia center having clinicians experienced both in VWD and in the coagulation laboratory that measures VWF and FVIII activities among other coagulation variables. It is desirable to manage and follow-up patients with VWD and other bleeding disorders with help of a computerized registry that includes relevant clinical and laboratory information. This is already introduced, or ongoing, in most countries/centers in the Nordic region according to the EUHANET policy. Patients with severe VWD (especially type 3), or those with frequent or severe bleeds should be observed once or twice a year or more often if required. Milder patients may be observed less regularly, e.g., with 2-3 years intervals. The comorbidities associated with bleeding tendency, such as hypertension, renal impairment and anemia should be monitored on a regular basis. Drugs that are impairing hemostasis should be avoided. A list of interfering drugs, with focus on platelet function, is found in the NHC Nordic guidelines for the diagnostics of inherited platelet disorders (76).
* All patients should be given an “identity” or “bleeder’s” card to be kept available, and inform about the bleeding disorder, the initial treatment in case of trauma or bleed and contact information to the hemophilia center. The patient file should provide a risk alert of the VWD diagnosis and emergency contacts to the hemophilia center.

**Prophylactic treatment with VWF concentrates**

* Long-term prophylaxis with a VWF concentrate should be tailored in VWD patients with a history of severe and frequent bleeds. Intracranial bleed and joint bleeds in type 3 VWD patients are the strongest indication for further prophylactic treatment. If nose bleeds, menorrhagia or gastrointestinal bleeds are severe and cause anemia despite iron supplementation and a major impact on social life, and/or other treatment modalities have failed, prophylactic replacement therapy should be provided. An international multicenter cohort study has shown that prophylactic treatment of VWD is efficacious (37).
* When used for prophylaxis in outpatients, a VWF concentrate at a dose of about 20-50 IU VWF:RCo/kg i.v. administered 2-3 times per week may be sufficient to prevent bleeds. Levels of VWF:RCo and FVIII:C should be monitored along the use of prophylaxis and in association of patient visits or invasive procedures.

**Management of patients with alloantibodies to VWF**

* Although very seldom, some type 3 VWD patients develop anti-VWF alloantibodies after multiple transfusions. Exposure to VWF containing products may cause life-threatening post-infusion anaphylaxis besides being ineffective.
* Recombinant FVIII can be given at large doses during surgery. Continuous infusion is mandatory due to the very short half -life of FVIII.
* By-passing therapy with activated prothrombin complex concentrates aPCC (FEIBA) and rFVII (NovoSeven) may also be considered.
* IV immunoglobulins prior to administering VWF may help to achieve critical levels of VWF and FVIII

**Acquired von Willebrand syndrome (AVWS)**

* Management of AVWS involves treatment of both bleeds and the underlying condition. VWF and FVIII levels can be raised either with desmopressin or with a VWF-FVIII containing concentrate, but factor levels can be short-lived due to increased clearance. Recombinant activated factor VII (rFVIIa) has been effective in some cases that were resistant to desmopressin or VWF-FVIII concentrates. Administration of high dose intravenous IgG (IVIG) may prolong the half-life of VWF by interfering with clearance mechanisms. IVIG has been used in connection with treatment of bleeds and for prophylactic treatment during surgery or delivery. However, IVIG is not working if the antibody is of IgM isotype. Plasma exchange has also been successful in some cases with a monoclonal antibody. Extracorporeal immunoadsorption has been reported in cases with high titer inhibiting antibodies. Immunosuppressive agents and corticosteroids are effective in some patients with autoimmune disorders or monoclonal gammopathy of undetermined significance (MGUS).
* Treatment of the underlying condition may result in improved or normalized VWF levels. Complete restoration has been achieved after tumor resection, chemotherapy, radiotherapy, valve replacement, or thyroxine replacement (54, 55).

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**Appendix**

Appendix 1: Products for the management of VWD