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

Guidelines of the Nordic Hemophilia Council

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Contents

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The Nordic Hemophilia Council

The Nordic Hemophilia Council (NHC) is a cooperative group of experts from the Nordic Hemophilia Centers. The NHC holds a general annual meeting and forms a base for co-operation between the Nordic centers. An executive committee of NHC is responsible for the management of the society's businesses such as implementation of the Nordic guidelines for bleeding disorders. The following haemophilia centers are found in the Nordic countries:

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Diagnostic guidelines

BACKGROUND OF VON WILLEBRAND FACTOR AND VON WILLEBRAND DISEASE

DEFINITION OF VON WILLEBRAND FACTOR AND VON WILLEBRAND DISEASE

VWF is a large multimeric protein with two main functions in hemostasis. It is responsible for the flow-dependent tethering of platelets to the subendothelium (adhesion) and bridging to other platelets (aggregation) securing platelet plug formation and primary haemostasis. Furthermore, VWF is a carrier protein for coagulation factor VIII (FVIII), which is thereby protected from degradation in plasma.

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 deficiency, 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 von Willebrand disease (VWD), due to the impaired primary haemostasis. These bleeds are due to non-optimal interaction between vessel wall and platelets, where the role of von Willebrand factor (VWF) is crucial.

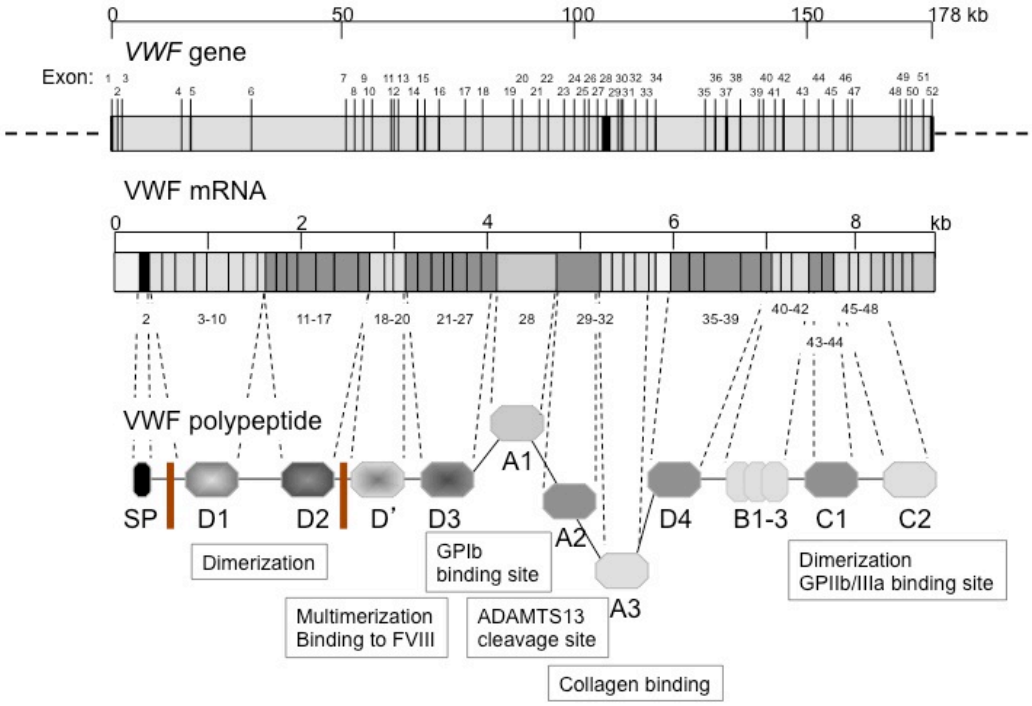

Figure 1. Schematic illustration of the structure and processing of the *VWF* gene, mRNA and polypeptide. The *VWF* gene covers 178 kb of DNA and is located on chromosome 12 and contains 52 exons. The gene is transcribed into an 8.8 kb mRNA that is translated to 2813 amino acid precursor polypeptide, 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. This propeptide is 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 a number of distinct functional domains, including binding to FVIII (domain D1 and D3), binding to platelet receptor GP1b (A1), binding to collagen (A1 and A3) and binding to platelet receptor GPIIb/IIIa (C2). A cleavage site for ADAMTS13, that regulates the size of the VWF multimers, is located 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 the healthy population there is a 5-fold variability of VWF levels and activities of which 20% is influenced by ABO blood group, 35% by genetics of VWF and 35% is due to unknown genetic background. VWF protein is secreted into plasma from the EC by a continuous constitutive secretion mechanism, while VWF in platelets is released only upon platelet activation. EC may 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 a molecular weights ranging from 500 - 20.000 kDa. The mature VWF protomer hosts several well-characterized binding sites. Most importantly, regarding VWD, one binding site interacts with collagen and another site with glycoprotein (GP) Ib of the platelet surface contributing to platelet adhesion at the wound site. This particular function in primary haemostasis depends 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, hereby 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 involved also 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 (also called triplet structure) that can be revealed by multimer analysis. The protective effect of FVIII by VWF is important for its survival of in circulation, since lack of VWF as well as 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 % in individuals with a normal secretion rate of FVIII.

A recommendation for the comprehensive nomenclature of protein functions and corresponding antigenic quantities has been adopted by the International Society on Thrombosis and Haemostasis (ISTH, Table 1).

Table 1. Recommended abbreviations for von Willebrand factor quantity and quality, as proposed by the von Willebrand Factor Scientific and Standardization Subcommittee of ISTH[1].

Attribute	**Recommended abbreviations**
Mature protein	VWF
Antigen	VWF:Ag
Ristocetin Cofactor activity	VWF:RCo
Collagen Binding capacity	VWF:CB
Factor VIII Binding capacity	VWF:FVIIIB

VON WILLEBRAND DISEASE (VWD)

From the aforementioned it is well understood that VWD is caused by a defect inflicting platelet function, and the major clinical hallmark in VWD is a tendency to mucocutaneous bleeds.

The history of VWD dates back to Erik von Willebrand[2] who reported on a bleeding disorder, with fatal outcomes, denoted pseudohemophilia, occurring equally often in both sexes. Today, our knowledge shows 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 and platelets. The basis for diagnosis of VWD relies on patient's and relatives' medical history, signifying an increased bleeding tendency together with a phenotype compatible with a defect of primary haemostasis.

Since many variants of VWD had been reported, the ISTH has developed a guideline for classification of VWD, simplifying the hierarchy of subclasses. Table 2 summarizes the current recommendation, including the amendments agreed upon during the ISTH VWF SSC meeting in Oslo, June 2006[3].

Table 2. Types of von Willebrand disease

Type	Description
1	Partial quantitative deficiency of VWF
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 without a selective deficiency of high molecular weight multimers
2N	Markedly decreased binding affinity for FVIII
3	Virtually complete deficiency of VWF

In many type 2 and 3 variants of VWD, genetic defects have been identified, while in many type 1 individuals 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 recent multicentre studies on type 1 VWD our knowledge of the complexities causing VWD have been extended (for a recent review on VWD genetics see ref. no. 4 by AC Goodeve) 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, such as a blood group O genotype or yet unidentified regulatory genes. The consensus today is, that the diagnosis of VWD should not depend on identification of a mutation in the VWF gene locus[3] but rather on the activity level. VWF gene mutations are listed in an international web based registry hosted by the University of Sheffield (http://www.vwf.group.shef.ac.uk/).

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, in particular if a history of mucocutaneous bleeding is present. Thrombocytopenia and platelet function defects, e.g. Bernard-Soulier, Glanzmann thrombasthenia, secretion disorders, as well as acquired causes of dysfunction (e.g. drugs, uremia or haematological disorders) also impair primary haemostasis. In the surgical setting the distinction must be made between a bleeding diathesis versus bleeding caused

by insufficient surgical haemostasis (check from the surgical files). A profuse generalized bleeding tendency is typical of a coagulopathy, as for example encountered in association with disseminated intravascular coagulation.

DIAGNOSIS OF VWD AND IT'S SUBTYPES

The diagnosis of VWD is based on three main criteria:

- the patient should have significant bleeding symptoms,
- there should be a family history of VWD or significant bleeding symptoms, except in recessively inherited subtypes, and
- VWF levels should be significantly decreased.

While the detection of VWD may be relatively simple, its classification by best standards requires a quite extensive laboratory armamentarium.

A clinician with special knowledge of coagulation disorders should make the diagnosis of VWD. General practitioners should refer the patient to a hematologist or a specialist working at the unit of coagulation disorders. The diagnosis should be confirmed and reassessed at least every 10 years, as ageing populations with bleeding disorders may be subject to overdoses of replacement therapy and, possibly, to the risk of thrombosis.

The diagnosis is based on the interaction between the clinician and the laboratory specialist, but the diagnosis should be clinical and based on bleeding score or relevant assessment of bleeding tendency, not just on a low laboratory value of VWF.

INITIAL SCREENING TESTS

The bleeding time (BT) is often prolonged but the sensitivity is low in mild forms of VWD. BT may be helpful as a screening test to identify moderately or severely low VWF when specific assays are not available, but has low sensitivity and specificity and does not differentiate from platelet defects. The result of the BT is operator-dependent and should only be handled by specially trained personnel on selected patients. A special point-of-care *in vitro* bleeding time device, the Platelet Function Analyzer-100® (PFA-100) may be helpful as a screening test to identify moderately or severely low VWF as well as some platelet function disorders. The instrument comes with two types of collagen-coated cartridges (epinephrine or ADP) and measures the closure time of a small aperture in an artificial collagen membrane. The closure time is dependent on the content of VWF in platelets and plasma. Similar to the BT test, however, the PFA-100 has low sensitivity for mild defects in primary hemostasis and lacks specificity since it does not differentiate VWD from other platelet defects.

The platelet count is usually normal with the exception of a mild to moderate thrombocytopenia found in patients with VWD type 2B or the platelet-type (pseudo) VWD. Global plasma tests such as activated partial thromboplastin time (APTT) and prothrombin time (PT), are often normal but the APTT may be prolonged in cases where FVIII level are below 0.30-0.40 kIU/L. The APTT may show considerable differences in factor sensitivities between reagents.

As shown by the algorithm in Fig. 2, the diagnosis of VWD is dependent on a significantly reduced ristocetin cofactor (VWF:RCo) activity, with the exception of type 2N. The Nordic Hemophilia Council recommends that the diagnosis of VWD should not be applied unless repeated VWF:RCo levels below 0.35 kIU/L are demonstrated. However, the exact level remains a matter of debate, and some individuals will be diagnosed with VWD also with somewhat higher VWF:RCo levels, dependent on symptoms and family history after exclusion of platelet defects. Measuring factor VIII:C level is important as a prerequisite to approach the subtype 2N diagnosis. Additionally, Factor VIII plays an important role in assessment of the bleeding and likely thrombosis risks in VWD. However, in all subclasses of VWD the level of factor VIII may be decreased, and it is particularly low (< 0.05 kIU/L) in type 3.

Table 3. Laboratory procedures commonly used for diagnosis and subclassification in von Willebrand disease.

Method	**Diagnostic and monitoring purposes**
Ristocetin Cofactor activity (VWF:RCo)	The main functional VWF method. Sensitive to loss of high multimer VWF and measures the ability of the VWF to bind GPIb and, hence, cause agglutination of platelets or latex particles coated with recombinant GPIb protein.
VWF antigen (VWF:Ag)	Quantitation of VWF antigen (protein). Used to differentiate between VWD type 1 and type 2.
Ristocetin induced platelet aggregation (RIPA) using patient's platelet rich plasma (PRP)	Detection of the sensitivity of platelets to ristocetin. Increased sensitivity at ristocetin concentration of 0.5 mg/ml or lower, is indicative of VWD type 2B. RIPA is absent in VWD type 3 and generally decreased in type 2A.
VWF collagen binding (VWF:CB)	Detects VWF multimer impairments as the VWF:RCo assay does but specifically assesses the collagen binding capacity of VWF. The assay is performed in the microplate (ELISA) format and the discriminatory power is dependent on type of collagen used.

VWF multimeric distribution and –pattern ▪ Gel concentration low to intermediate ▪ Gel concentration high	Electrophoretic procedure essential for detection of VWF multimeric size distribution ▪ Differentiation of type 1 VWD from type 2A ▪ Identification of type 2M (full range of multimers) ▪ Study of the oligomeric structure and further subclassification (in variants such as type IIA, IIB, IIC, IID etc.).
FVIII binding capacity of the VWF (VWF:FVIIIB)	Determines the capacity of VWF to bind FVIII. Specific test for VWD type 2N.
FVIII coagulation activity (FVIII:C)	Determination of FVIII coagulation activity. A disproportionally reduced FVIII (compared to VWF) is found in VWD type 2 N.

COMMENTS REGARDING LABORATORY METHODS

Ristocetin cofactor activity (VWF:RCo)

This functional method is the key diagnostic test and is based on agglutination of normal platelets by patient plasma in the presence of the antibiotic ristocetin. Agglutination is conventionally measured in a platelet aggregometer as a change in optical density. The method is quite laborious, its performance being highly dependent on the quality of the platelets, and the accuracy and reproducibility of VWF:RCo varies. In recent years, several fully automated VWF:RCo assay protocols have been developed. These utilize common coagulation analyzers instead of the manually operated aggregometers. They allow greater throughput of samples with improved analytical precision compared with manual and semi-automated assays[5, 6]

The test is a marker for the adhesive function of VWF and explores the interaction of VWF with platelet receptor GPIb complex. Although VWF:RCo represents a non-physiological measurement of the capacity of VWF to interact with platelet GPIb, it correlates well with clinical phenotype, as the assay is sensitive to functional high molecular weight multimers. Thus, the assay is likely to be abnormal in all types of VWD except for type 2N.

Other assays that assess the VWF-GPIb interaction exist. Pure immunobinding assays, independent of ristocetin, are based on monoclonal antibodies directed against the functional epitope of VWF with the binding site for GPIbα. These can be performed by ELISA or as a fully automated latex immunoassay. Novel, ristocetin independent assays, that only utilize GPIb have also been published. These utilize gain-of-function mutated GPIb constructs that bind VWF without need of any modulator. These ristocetin-independent assays are unaffected by common polymorphisms found in the black American population that may

result in false low VWF:RCo results. There are now commercial variants of this assay, one comprising a latex particle enhanced agglutination assay from Siemens (Innovance VWF Ac) that is easy to perform on common coagulometers with apparently good reproducibility and sensitivity. Thus, VWF:RCo 'alternative' assays may measure binding to GPIb, or fragments thereof, directly or indirectly though specific antibodies and with or without ristocetin as modulating agent. Currently, these activity assays have not been extensively validated and cannot be recommended to replace VWF:RCo in clinical routine.

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 as automated latex-enhanced immunoturbidometric assays performed on coagulation analyzers. Both assay principles have the same diagnostic capacity but the reproducibility is better using automated assays[7].

Normal amounts 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 present with quite normal quantities of antigen, which is however dysfunctional.

Ristocetin-induced platelet aggregation (RIPA)

This test determines the 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 from the patients it is necessary to perform the test within 2 h of blood collection.

This method is relatively insensitive to quantitative deficiencies of VWF, but serves an important diagnostic role since type 2B variants display increased platelet aggregation to low concentrations of ristocetin. There is consensus that increased sensitivity to ristocetin at 0.5 mg/ml or lower indicates the presence of type 2B VWD. Normal individuals will show platelet aggregation at and above 1.0 mg/ml but typically not below this concentration. 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.

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. Several commercially test kits are available. The collagen is immobilized in wells of an ELISA plate and is used to catch VWF in plasma. After washing, any bound VWF is detected using an enzyme-conjugated anti-VWF antibody. The source and type of collagen used in the assay are crucial and results may differ between different manufacturers that have different collagen formulations in their kits. In general, type I collagen binds VWF poorly whereas type III binds VWF so well that type VWD 2A variants give results comparable to the VWF:Ag assay. Thus, an assay based on a mixture of 95% type I and 5 % type III collagen is to be preferred as it has shown to give the best discrimination of VWD subtypes[8, 9]. The ELISA format gives technical advantages in its robustness, reagent stability and availability but its cost-effectiveness is greatly reduced if only few samples are analyzed on each test occasion.

The method reflects a biological function of VWF and contributes to the diagnosis of VWD by providing information about the quality of VWF. Both VWF:CB and VWF:RCo assays show positive correlation with the multimeric structure of VWF and can be equally used to classify VWD type 2, with the exception of type 2M, which is usually normal with the VWF:CB method. The VWF:CB assay should not be used to replace the VWF:RCo assay but rather used as a supplementary phenotyping assay. The approach to use a combination of VWF:RCo and VWF:CB assays has been shown to reduce diagnostic errors in clinical laboratories[10].

Multimeric sizing electrophoresis techniques

The study of VWF multimer composition is based on electrophoresis of plasma in a gel system suited for separation of macromolecules. Following separation, VWF molecules are electroeluted onto an immobilizing membrane on which patterns of VWF multimeric subsets are identified by means of an immuno-enzymatic or lumographic technique. The test is cumbersome, and difficult to perform and interpret and therefore should be performed only in specialized laboratories with long-term experience. 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. However, there is lack of consensus on the definition of areas to quantitate; a suggestion is to divide the multimers into small (multimer bands 1-5), intermediate (6-10) and large (>10). It is also possible to adapt the method by changing the gel system in order 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[11].

In VWD type 1 all multimers are present whereas VWD type 3 is characterized by complete loss of all multimers. In VWD type 2A there is often a severe loss of large and intermediate multimers. Also most type 2B patients display a loss of large multimers but exceptions, with normal multimers have been reported. Patients with VWD type 2M demonstrates all multimers, sometimes larger than normal (supranormal), but the triplet structure shows a decrease of subbands resulting in a blurred ("smeary") appearance. The multimers may also be absent of satellite bands in some type 2M patients. An aberrant multimer pattern may also be seen in type 2N due to abnormal disulphide bonds in the VWF molecule but the method cannot be used diagnostically in this subtype.

von Willebrand factor binding to FVIII (VWF:FVIIIB)

This test is indicated if coagulation assessment reveals a low FVIII level in a patient with a negative family history of hemophilia, where a low FVIII may result from a decreased carrier effect of VWF. In principle, an assay is performed in which 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 is determined by a chromogenic FVIII assay, or by an antibody to FVIII.

The VWF:FVIIIB is an important test for correct classification of VWD type 2N and it is also used to differentiate between type 2N VWD and hemophilia A.

von Willebrand factor propeptide (VWFpp)

The VWF propeptide separates from the VWF precursor after secretion of VWF to the circulation. The propeptide 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). In such cases the ratio of VWFpp/VWF:Ag is high as VWF but not the propeptide is cleared rapidly.

Anti-VWF antibodies

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:RCo activity is measured and compared with a control of pooled normal plasma mixed with VWF-deficiency plasma. It is also possible to measure antibodies against VWF using the ELISA technique. The ELISA cannot discriminate between neutralizing and non-neutralizing 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. The method can also be used to detect autoantibodies to VWF associated with acquired von Willebrand syndrome.

PRACTICAL DIAGNOSTIC CONSIDERATIONS

Pre-analytical considerations

Blood samples should preferably be collected in the coagulation laboratory. The patient should be at rest. Exercise, stress, infections and pregnancy elevate VWF and FVIII levels and may obscure the diagnosis of mild VWD type 1. Care should be taken to remove platelets from plasma by centrifuging to prevent platelet von Willebrand factor from mixing with plasma on freezing and thawing the sample. Some laboratories do this using double centrifugation. If not tested immediately, plasma samples should be frozen without delay at -70°C. If transportation of plasma samples is needed, samples should be kept frozen. If not frozen there is a risk of milk box misdiagnosis: if cooling of the plasma sample occurs during transportation, VWF:RCo and FVIII activities decrease. Blood can be drawn without consideration to the menstrual cycle[12, 13].

Laboratory tests in suspected VWD

Screening tests: Complete blood count with differential analysis and platelet count, prothrombin time (PT), activated partial thromboplastin time (APTT), VWF:RCo, VWF:Ag, Factor VIII level, ABO blood type. In urgent situations the use of PFA-100 may help excluding moderate and severe forms of VWF. Prolongation of the closure times, however, is not specific for VWD and may also indicate thrombocytopenia, platelet dysfunction or antiplatelet agents.

Defining the subtype of VWD: A ratio of VWF:RCo/VWF:Ag <0.7 defines type 2 VWD and RIPA and VWF multimer and/or VWF:FVIIIB further defines the type 2 subtype. Mutational analysis may also help to define the VWD subtype. Additional use of the a VWF:CB will reduce potential errors in VWD misidentification and may add information in the differentiation between 2A and 2M subtypes.

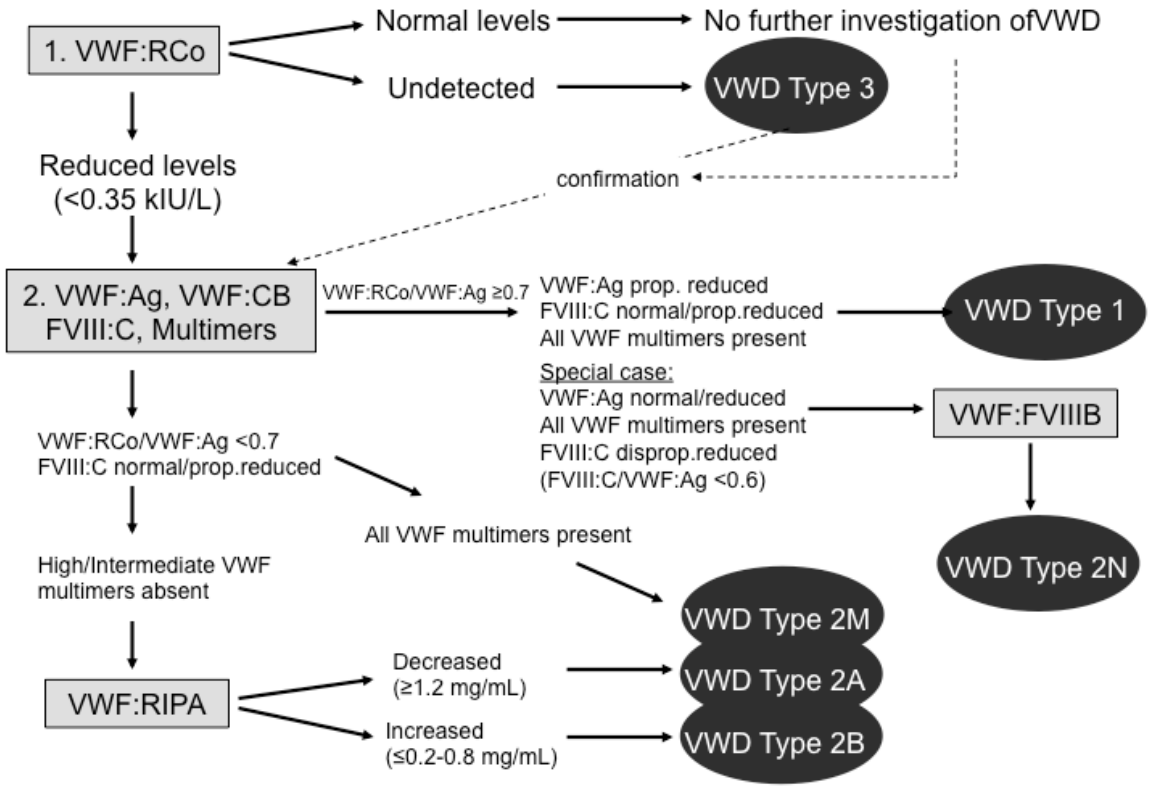

Figure 2. Example of a laboratory algorithm for investigating suspected von Willebrand disease. The thresholds given for VWF:RCo/VWF:Ag and FVIII:C/VWF:Ag ratios and the VWF:RIPA assay are not absolute and must be interpreted in the context with the patient's clinical history and other laboratory findings. 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 lead to an increased diagnostic error rate.

DDAVP test

All patients with type I and type 2A VWD should be given a test dose to ensure that the response is sufficient for clinical use. The recommended test dose is 0.3 microgram/kg intravenously or subcutaneously. The intravenous dose can either be given as a slow infusion of DDAVP dissolved in 10-15 ml saline over 15 minutes or as an infusion of DDAVP dissolved in 50-100 ml saline and infused over 30 minutes. Blood samples for VWF and FVIII measurements should be taken before and 30-60 minutes after the start of the intravenous dose and 60-120 minutes after the subcutaneous dose. A further blood sample taken after 4 hours is advisable to exclude that the patient has a very short half-life of released VWF and/or FVIII following DDAVP stimulation.

Genotyping and DNA samples

DNA analysis is currently not a routine application. However, some coagulation centers provide DNA analysis in order to confirm the laboratory phenotype of VWD type 2 (A, B, M or N). This is possible as there are a limited number of exons involved in which mutations causing qualitative VWD variants can be found. It is also possible to search for a specific mutation of any VWD type in family members with a known disease-causing mutation in the VWF gene. However, today it is not justifiable to genotype VWD type 1 and type 3 as the gene is large and mutations can be found in any position of the VWF gene. Mutations causing VWD and polymorphisms of the VWF gene can be found in a database on Internet (www.vwf.group.shef.ac.uk) that is maintained by the VWF subcommittee of International Society on Thrombosis and Haemostasis (ISTH).

It would be advantageous for future genetic studies and family investigations if blood samples for mutation analysis were taken in patients diagnosed with VWD, and stored for possible mutation analysis at a specialized laboratory. Approval for storage of blood samples in a bio-bank must be gathered from the patient and from the appropriate authorities.

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. Joint bleeds and frequent gastrointestinal bleeds occur in severe cases. 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).

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 its correlation with clinical and laboratory features[14]. The BS showed a strong significant inverse relation with VWF:RCo, VWF:Ag or factor 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) [14]. ISTH has taken initiative to develop 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) [15]. This kind of BS may be a useful screening tool for VWD and it´s use is encouraged at the Nordic Hemophilia Centers, see appendix 1. A version, better suited for pediatric patients, was published in 2009 [16].

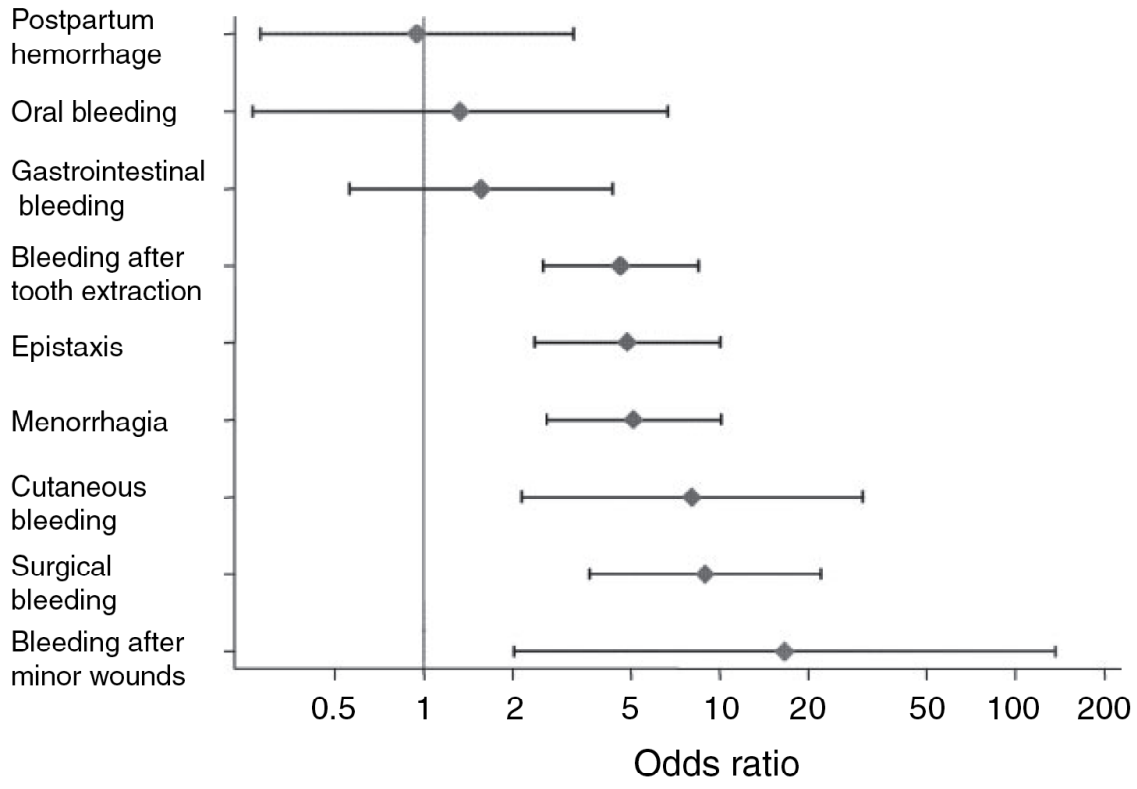

Figure 3. Symptoms strongly suggestive of VWD. Association between bleeding symptoms and type 1 VWD in enrolled families in an age-adjusted logistic model is shown. Index cases are

excluded from the analysis. The graph reports the logistic estimate and its 95 % confidence interval (from Tosetto et al., 2006[14]).

CRITERIA FOR FAMILY HISTORY

A positive family history compatible with VWD (except for types 2N and 3) requires that at least one first-degree relative, or at least two second-degree relatives, have a personal history of 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 permit the investigation of more remote relatives, and may allow asymptomatic relatives with low VWF levels to provide evidence for inheritance.

CRITERIA FOR VWD TYPE 1

Laboratory test results are considered compatible with VWD type 1 if the levels of both VWF:RCo and VWF:Ag are < 0.35 kIU/L on ≥2 determinations. Additionally, if performed, RIPA must not indicate abnormal sensitivity to low concentrations of ristocetin, and the plasma VWF multimer distribution must be normal.

Type 1 VWD: VWD type 1 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, laboratory tests compatible with VWD type 1, and either a positive family history for VWD type 1 or an appropriate VWF mutation.

Possible type 1: VWD: Possible VWD type 1 includes persons with laboratory tests compatible with VWD type 1 and either significant mucocutaneous bleeding or a positive family history for VWD type 1.

To meet this definition, an asymptomatic person with low VWF must have a positive family history, which means that they must have at least two relatives with definite VWD type 1. Asymptomatic individuals are typically children who have not yet been challenged with trauma or invasive procedures that could cause bleeding.

In many circumstances, symptomatic patients with either VWD type 1 or possible VWD type 1 will be treated identically. Such empiric treatment may also be appropriate for selected asymptomatic patients. The distinction between possible VWD type 1 and definite VWD type 1 will be useful for certain clinical studies and genetic studies. Alternative or additional diagnoses should be re considered for patients with possible VWD, including the co-existence of platelet disorders.

Special considerations on the diagnosis of type 1 von Willebrand disease

In the investigation of type 1 VWD, the bleeding history is particularly important. The relatative weight 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[14].

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[4]. 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 marginally low levels of VWF and equivalence between VWF:RCo 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 >0.45 kIU/L[9]. Recent work has further demonstrated an inverse relationship between VWF mutations and levels of VWF in these patients. "Genetically confirmed" VWD type I has a high diagnostic sensitivity only at VWF:Ag and VWF:RCo levels below 0.35 kIU/L (Fig 3), which seems a reasonable diagnostic cut-off level. This observation is further concordant with the opinion of the working group on Classification of von Willebrand disease of ISTH, SSC[3]. However, it should be stressed that marginally low VWF levels not qualifying for VWD criteria are associated with increased mucocutaneous bleeding as shown in two case-control studies[13,17] and that many such individuals actually also have VWD associated mutations[18].

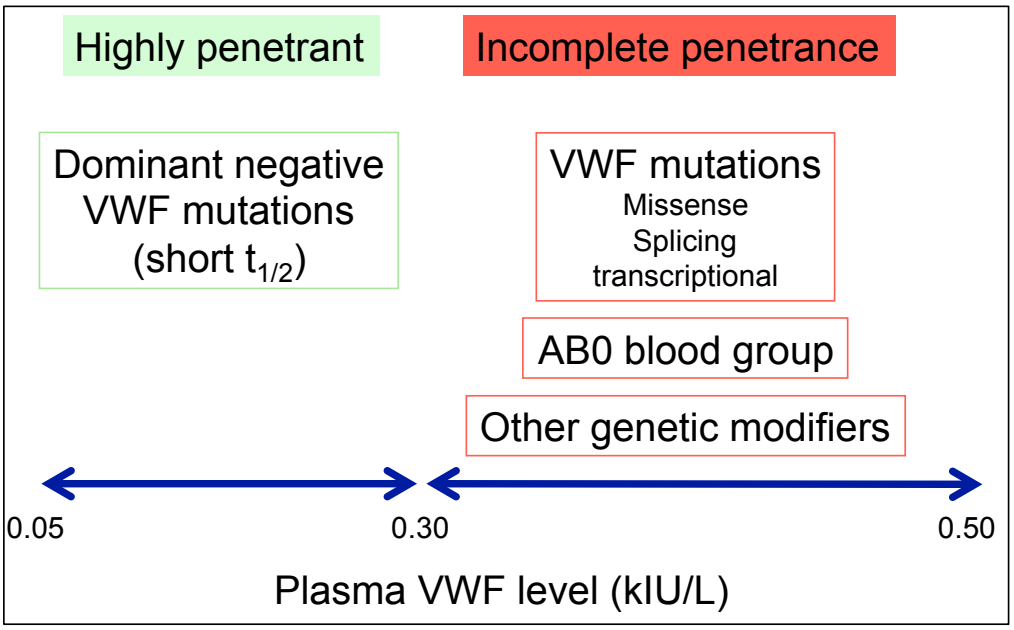

Fig. 4. Schematic model that illustrates factors influencing the VWF levels in plasma and the relationship with the heritability (modified from ref. no. 19).

The presence of blood group O generally predicts lower levels of VWF compared with the non-O state, and blood group O itself represent a risk factor for lowered VWF with 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. 0.35-0.50 U/ml), but rather to denote symptomatic persons with VWF in that range as having a mild bleeding disorder or a risk factor for excessive bleeding.

CRITERIA FOR VWD TYPE 2

VWD type 2 is defined by low levels of functional VWF:RCo and a low VWF:RCo/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. The 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 [20]. 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[21].

Also, 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[23, 24].

Type 2B is caused by mutations in the A1 domain, and is characterized by an increased sensitivity to ristocetin in the RIPA test. In the classical form of type 2B, there is a lack of HMWM and a tendency to thrombocytopenia. Thrombocytopenia may be worsened by pregnancy, infections, stress or administration of DDAVP. In the rare Type 2B Malmö/New York [25], the multimers are normal and there is no tendency to thrombocytopenia. This variant resembles type 1, apart from the increased RIPA.

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. Mutations are in the D3 domain[26].

FVIII deficiency is the typical feature of type 2N VWD due to FVIII binding defect to VWF, and FVIII ranges from 0.01 – 0.40 kIU/L, but is usually above 0.05 kIU/L[27]. In some patients discrepant FVIII levels are traced by different FVIII:C methods (one-stage, two stage or chromogenic methods). 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, but it may be only slightly decreased in compound heterozygotes for a type 2N mutation and a mutation causing a quantitative VWF deficiency. A low VWF:FVIIIB/VWF:Ag ratio seems to predict type 2N the best[28].

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 (about 0.05 kIU/L). It is very rare, with a prevalence of about 2-3 cases per million in the Nordic area. Higher figures can be seen in countries with high degree of consanguinity. Bleeding symptoms are usually moderate to severe, and many type 3 patients require substitution therapy with VWF-containing concentrates. The patients are usually homozygotes or compound heterozygotes for mutations in the VWF gene[4]. Bleeding symptoms are more prevalent in obligatory carriers than in the normal population, but not as frequent as in patients with type 1 VWD[29].

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. About 50-60% of cases are caused by lympho- or myeloproliferative disorders. Other causes include solid tumors, immunological disorders, cardiovascular disorders, hypothyroidism, drugs and miscellaneous causes. The pathophysiology is not always clear. In hypothyroidism, synthesis of VWF is decreased. In most other conditions, synthesis seems to be normal, but clearance is increased through four different possible mechanisms, specific autoantibodies, non-specific autoantibodies forming immune complexes, adsorption to malignant cell clones or increased proteolytic degradation[30]. 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. Laboratory findings usually include mildly decreased VWF:Ag and FVIII:C but more pronounced decrease of VWF:RCo and VWF:CB. The VWF:RCo/VWF:Ag ratio is therefore frequently decreased. The large multimers are often lacking due to increased clearance. Thus, these patients often exhibit a type 2A resembling phenotype. Mixing of patient plasma with normal plasma seems to be a relatively insensitive method to identify inhibiting antibodies. An ELISA assay appears to be more sensitive than functional assays. VWF propeptide levels may be increased in plasma, but the assay is not generally available. An international registry collects data about this rare disorder[31].

Guidelines on treatment and management of VWD

INTRODUCTION

In VWD, bleeding tendency is caused by decreased levels or 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 mild type 1, or by substitution with a VWF/FVIII concentrate in type 2 and 3. DDAVP may temporarily normalize haemostasis if functional levels of VWF and FVIII can be reached by endogenous release. VWF/FVIII or purified VWF concentrates are used when DDAVP is not an alternative[32, 33]. Plasma-derived concentrates carry a potential risk of transmission of infectious agents, even if this risk is thought to be negligible with the current safety procedures, including sterilization. Cost is another issue, as these concentrates are expensive. On the other hand, safety may be compromised with DDAVP due to its side effects, mainly caused by the strong antidiuretic mechanism that may limit its use and also limit the duration of the treatment period. Antifibrinolytic treatment (tranexamic acid) is important adjuvant to DDAVP or concentrates, or sometimes as a single haemostatic agent, especially in connection with mucous membrane bleeds. Oral contraceptive pills may be used as treatment of menorrhagia in females with VWD, as contraceptives typically decrease menstrual blood loss. Progesterone releasing IUDs are highly effective in some women with menorrhagia[34].

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 – one dose 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 children and increased risk of thrombotic complications in elderly or in patients with strong cardiovascular risk factors.
- presence of other diseases that may contraindicate use of a therapeutic agent
- pregnacy

HAEMOSTATIC AGENTS

The different haemostatic 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

Suggested treatment options are given below and summarized in appendix 2.

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 haemostatic effect is achieved with a dosage of 0.3 microgram/kg given intravenously. A higher dose will not improve the response[35]. 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 microgram/kg i.v. or s.c.
- 300 microgram i.n. (spray) (150 microgram if BW<30 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 microgram/mL) is the most suitable for s.c. administration, due to its high concentration. Often a single 15 microgram 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 microgram. For patients with a body weight <30 kg, a dose of 150 microgram is recommended (one spray in one nostril). In small children (less than about 15 kg) the spray should not be used.

TEST DOSE (BIOLOGICAL RESPONSE)

Most patients should be given a test dose to ensure sufficient response for clinical use. Testing children is usually omitted until the age of 4 years. Blood samples for VWF and FVIII measurements should be collected before and after 30-60 minutes. It is advisable to collect a further blood sample after 4 hours to exclude a very short half-life of VWF and/or FVIII after DDAVP.

DOSAGE INTERVALS

Twelve to 24 hours is the ordinary dose interval, but depending on the half-life of factor levels and the nature of the bleed, the dose may be given with 8-hour intervals in hospitalized patients. 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 intake is 1.5 liter per day of administration.

TREATMENT WITH DESMOPRESSIN RELATED TO THE NATURE OF THE BLEEDING EPISODE

Major bleeds

Response criteria:

DDAVP can be used for treatment of major bleeds in patients in whom the administration leads to normal VWF:RCo and FVIII:C levels . The levels should remain at least above 0.30 kIU/L during 12 hours post DDAVP (Consensus opinion. No data). In connection with life threatening, and other severe, bleeds a VWF/FVIII concentrate should be administered as the first line treatment.

If the response 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 0.30 kIU/L within 2 hours after DDAVP.

Type of invasive procedure

All procedures – VWF and FVIII activities should reach normal levels within the first 2 hours and stay elevated ≥ 0.30 kIU/L for at least 12 hours post DDAVP.

If treatment ≥ 3 days is required, anaphylaxis and antidiuretic effects must be taken into account, and factor levels and sodium should be monitored.

Treatment varies with the procedure and more options at different procedures are given in appendix 2.

LIMITATIONS

- Consider the subtype of VWD, duration of treatment and age of the patient.
- 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.
- Half-life of FVIII:C may be short in 2N.
- Half-life of VWF:RCo may be short in certain subtypes.
- DDAVP should preferably not be given to small children (<2 years, elective testing 4 years), and adult patients with cardiovascular disease or comorbidities (e.g. history of angina, AMI, stroke, arrhythmias, epilepsy). If DDAVP is administered to young children, fluid administration must be restricted and electrolytes monitored closely.
- If repeated doses are given 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. Tachyphylaxis has been reported after several days of treatment with DDAVP.
- Pregnancy

ADVERSE EFFECTS

Tiredness, headache, nausea, decreased appetite, temporary lowering of blood pressure with secondary tachycardia, facial flushing, fluid retention, hyponatremia and seizures

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.

DOSE AND MODES OF ADMINISTRATION

Orally 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

- 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 or family history of thromboembolic disease.
- No data are available on the use of tranexamic acid in newborns.

ADVERSE EFFECTS

Nausea, vomiting, diarrhea and abdominal pain.

ORAL CONTRACEPTIVE PILLS OR PROGESTERONE IUD

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.

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 more or less 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 the purity.

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 haemostasis. 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. A perfusion chamber technique with flowing blood would be preferable to measure the platelet and collagen binding functions of VWF, but such methods are not generally available, and not standardized.

The 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). A concentrate with a high relative amount of FVIII (a low VWF:RCo/FVIII:C ratio) may cause high plasma levels of FVIII, 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 very high FVIII levels. 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

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 0.015 – 0.020 kIU/L per infused IU VWF:RCo/kg body weight. A dose of 50 IU/kg can be expected to increase the VWF:RCo with about 0.75 – 1.00 kIU/L. 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. A table with suggested dosing of VWF/FVIII concentrates at various clinical situations is given in appendix 3.

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 may be 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[35].

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, available in Denmark, Norway and Finland. (under registration in Sweden). A plasma-derived concentrate with a VWF:RCo/FVIII:C ratio of approx. 60. This is to be considered if the patient and/or the invasive procedure have strong thrombogenic properties to avoid exogeneous additive FVIII .

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, a 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 vitamin A containing drops or 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. 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. The respective dosage levels are specified above. All local procedures fostering primary haemostasis, such as fibrin glue are recommended.

MENORRHAGIA

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 week, in some cases only during the first days of menstruation. If tranexamic acid and oral contraceptives are not sufficient, DDAVP or a VWF concentrate may be 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) bleeds may have many different causes, both acute or chronic and benign or malignant. The source of the bleed may be located anywhere in the GI tract. In VWD, the dysfunctional primary haemostasis contributes to the tendency to bleed from angiodyplasias. These leading to GI bleed have been described in VWD since 1967. The prevalence of angiodyplasias in VWD is between 1.1-6.5 %. In VWD patients older than 50 years, they may be as prevalent as 10 % and occur both in patients with congenital and acquired VWD. There seems to be an association between Heyde's syndrome (aortic stenosis associated with bleeds due to GI angiodysplasia) and acquired VWD type 2A, with loss of the largest multimers of von Willebrand factor (VWF). An interesting observation is that aortic valve replacement generally cures GI bleed or its tendency in these patients, and that the deficient multimeric pattern of the VWF is normalized after the valve replacement. VWD patients with angiodyplasias may have recurrent severe GI bleeds requiring hospital admissions, and transfusion with packed red cells, FVIII or VWF concentrates and plasma. Various local invasive procedures have been tested, e.g. surgery, electrocoagulation, laser photocoagulation, sclerotherapy, coiling, and arteriography with embolization. However, these measures are often insufficient to stop recurrence of the GI bleeds. There are some case reports of good response to estrogens and progesterone, and beta-blocking drugs. In case of recurrent GI bleeds, secondary prophylaxis with a VWF/FVIII concentrates in a dosage of about 50 IU VWF:RCo/kg i.v. 2-3 times per week or even more frequent, in combination with oral tranexamic acid may be needed for short or long-term.

SURGERY AND OTHER INVASIVE PROCEDURES

Surgery and other invasive procedures should be performed at a specialized hemophilia center with clinicians experienced in VWD, and with a coagulation laboratory that can measure VWF and FVIII activities 24 h per day. Both FVIII and VWF levels must be taken into account, depending on the type of procedure. 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 7-10 days. Active VWF is needed to cease mucous membrane bleeds and VWF should be normalized in connection with invasive procedures involving mucous membranes. 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. Repeated infusions of a VWF/FVIII concentrate may induce high levels of FVIII:C in plasma, due to the additive effect of the endogenously released FVIII. Very high FVIII:C levels (>1.50 kIU/L) or VWF:RCo (>2.00 kIU/L) 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.

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 s. In some cases a combination of DDAVP and a VWF concentrate may be useful. DDAVP may be administered intranasal by 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 microgram i.n. (150 microgram 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 in a dose of 0.3 microgram/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 and surgery

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 0.015 – 0.020 kIU/L 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.

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 given in 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.

Thrombosis prophylaxis

Thrombosis prophylaxis should not be given routinely to patients with VWD undergoing surgery. It may be considered in patients with multiple prothrombotic risk factors in association with high doses of a VWF/FVIII concentrate.

PREGNANCY AND DELIVERY

During pregnancy the haemostatic system shifts to a prothrombotic direction. Plasma levels of VWF and FVIII increase significantly and may even normalize in patients with mild type 1 VWD. In type 3 VWF and FVIII do not increase and prophylactic treatment with VWF concentrates should be considered during pregnancy and delivery. Tranexamic acid is the first treatment option in case of mucocutaneous bleeds.

Generally, vaginal delivery is regarded as safe if VWF:RCo is > 0.40 kIU/L whereas for caesarean section the VWF:RCo level should be > 0.50 kIU/L.

The delivery should preferably take place at an obstetrical unit close to the hemophilia center. The hemophilia center should be involved early in pregnancy and should present a plan for the haemostatic treatment in connection with delivery in cooperation with the obstetrician.

In all cases, treatment with tranexamic acid is commenced when the patient arrives to the maternity ward to give birth, and thereafter continued until two weeks post partum. The dose is either 10 mg/kg i.v., or 20-25 mg/kg orally, every 6-8 hours.

In mild cases (DDAVP responders), DDAVP is administered intravenously in a dose of 0.3 microgram/kg immediately after delivery. One dose is usually sufficient, but DDAVP may be repeated with 8-12 h intervals if needed. In such cases, the risk of water retention must be remembered.

In DDAVP non-responders, treatment with a VWF concentrate is initiated when the delivery starts. A dose of 50-60 IU VWF:RCo/kg i.v. is recommended for patients with very low levels of VWF:RCo. In severe cases, treatment with a VWF concentrate about 3 times weekly, is continued over a period of two weeks postpartum. If FVIII levels are high despite low VWF levels a concentrate devoid (or with least possible amount) of FVIII should be prioritized.

Newborn babies with a family history of VWD but without bleeding symptoms are not routinely investigated for VWF and FVIII levels during the postnatal period as VWF and FVIII levels are raised postpartum and the diagnosis can thus be missed.

Bleeding symptoms are rare in newborns with VWD but can be seen after a traumatic delivery, especially in patients with type 3 and in these cases VWF and FVIII should be analyzed.

MANAGEMENT OF OUTPATIENTS

Patients with VWD should regularly visit a hemophilia center having clinicians experienced in VWD and a coagulation laboratory that can measure VWF and FVIII activities. 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/centres in the Nordic region. Patients with severe VWD (especially type 3), or those with frequent or severe bleeds should be seen once a year or more often if required. Milder patients may be observed less regularly, e.g. with 2-3 years intervals.

All patients should be given an "identity" or "bleeder's" card informing about the bleeding disorder, the initial treatment in case of trauma or bleed and contact information to the hemophilia center.

PROPHYLACTIC TREATMENT WITH VWF CONCENTRATES

Regular prophylactic treatment with a VWF concentrate may be considered in some patients. Joint bleeds in type 3 VWD patients is a strong indication for further prophylactic treatment. If nose bleeds, menorrhagia or gastrointestinal bleeds are so severe that they cause significant anemia despite iron supplementation and a major impact on social life and/or all other treatment modalities have failed prophylactic treatment should be provided. It has been concluded from an international multicentre cohort study 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 when repeated doses are administered over a longer period.

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 postinfusion 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.

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 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. Plasma exchange has been successful in some cases with a monoclonal protein. 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[30, 31].

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APENDIX

1) Bleeding score for adults

2) Treatment options

3) Treatment with VWF/FVIII concentrates at different clinical situations.