GUIDELINES

von Willebrand disease (VWD): evidence-based diagnosis and management guidelines, the National Heart, Lung, and Blood Institute (NHLBI) Expert Panel report (USA)[1]

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Summary. von Willebrand disease (VWD) is a commonly encountered inherited bleeding disorder affecting both males and females, causing mucous membrane and skin bleeding symptoms, and bleeding with surgical or other haemostatic challenges. VWD may be disproportionately symptomatic in women of child-bearing age. It may also occur less frequently as an acquired disorder (acquired von Willebrand syndrome). VWD is caused by deficiency or dysfunction of von Willebrand factor (VWF), a plasma protein that mediates platelet haemostatic function and stabilizes blood coagulation factor VIII. The pathophysiology, classification, diagnosis and management of VWD are relatively complex, but understanding them is important for proper diagnosis and management of patients with VWD. These evidence-based guidelines for diagnosis and management of VWD from the National Heart, Lung, and Blood Institute (NHLBI) Expert Panel (USA) review relevant publications, summarize current understanding of VWD pathophysiology and classification, and present consensus diagnostic and management recommendations based on analysis of the literature and expert opinion. They also suggest an approach for clinical and laboratory evaluation of individuals with bleeding symptoms, history of bleeding or conditions associated with increased bleeding risk. This document summarizes needs for further research in VWF, VWD and bleeding disorders, including clinical research to obtain more objective information about bleeding symptoms, advancements in diagnostic and therapeutic tools, and enhancement in the education and training of clinicians and scientists in bleeding and thrombotic disorders. The NHLBI Web site (http://www.nhlbi.nih.gov/guidelines/vwd) has a more detailed document, a synopsis of these recommendations, and patient education information.

Introduction

von Willebrand disease (VWD) is an inherited bleeding disorder that is caused by deficiency or dysfunction of von Willebrand factor (VWF), a plasma protein that mediates the initial adhesion of platelets at sites of vascular injury and also binds and stabilizes blood clotting factor VIII (FVIII) in the circulation. Therefore, defects in VWF can cause bleeding by impairing platelet adhesion or by reducing the concentration of FVIII.

VWD is a relatively common cause of bleeding, but the prevalence varies considerably among studies and depends strongly on the case definition that is used. VWD prevalence has been estimated in several countries on the basis of the number of symptomatic patients seen at haemostasis centres, and the values range from roughly 23 to 110 per million population (0.0023–0.01%) [1].

The prevalence of VWD has also been estimated by screening populations to identify persons with bleeding symptoms, low VWF levels, and similarly affected family members. This population-based approach has yielded estimates for VWD prevalence of 0.6% [2], 0.8% [3] and 1.3% [4] – more than two orders of magnitude higher than the values arrived at by surveys of haemostasis centres.

The discrepancies between the methods for estimating VWD prevalence illustrate the need for better information concerning the relationship between VWF levels and bleeding. Many bleeding symptoms are exacerbated by defects in VWF, but the magnitude of the effect is not known. For example, approximately 12% of women who have menstrual periods have excessive menstrual bleeding [5]. This fraction is much higher among women with VWD, but it also appears to be increased for women who have VWF levels at the lower end of the normal range. Quantitative data on these issues would allow a more informed approach to the diagnosis and management of VWD and could have important implications for medical practice and for public health.

In addition to the need for better information about VWD prevalence and the relationship of low VWF levels to bleeding symptoms or risk, there are needs for enhancing knowledge and improving clinical and laboratory diagnostic tools for VWD. Also needed are better knowledge of and treatment options for management of VWD and bleeding or bleeding risk. As documented in this VWD guidelines publication, published studies are lacking to support some of the recommendations that, therefore, are based mainly on Expert Panel opinion.

Guidelines for VWD diagnosis and management, based on the evidence from published studies, the opinions of experts, or both, have been published for practitioners in Canada [6], Italy [7] and the United Kingdom [8,9], but not in the United States. The VWD guidelines from the US Expert Panel are based on review of published evidence as well as expert opinion. Users of these guidelines should be aware that individual professional judgment is not abrogated by recommendations in these guidelines.

These guidelines for diagnosis and management of VWD were developed for practicing primary care and specialist clinicians — including family physicians, internists, obstetrician–gynaecologists, paediatricians, and nurse practitioners — as well as haematologists and laboratory medicine specialists. The National Heart, Lung, and Blood Institute (NHLBI) Web site [10] has a synopsis of these recommendations, patient education information, and a more detailed document that includes additional background information. Also on the Web site are 13 evidence tables that summarize published information supporting recommendations that are graded as B or higher and have two or more references.

Project history and methods

During spring 2004, the NHLBI began planning for the development of clinical practice guidelines for VWD. In consultation with the American Society of Hematology, the institute convened an Expert Panel on VWD, chaired by William L. Nichols MD. The Expert Panel members were selected to provide expertise in basic sciences, clinical and laboratory diagnosis, evidence-based medicine, and the clinical management of VWD, including specialists in haematology as well as family medicine, obstetrics and gynaecology, paediatrics, internal medicine and laboratory sciences. The Expert Panel comprised one basic scientist and nine physicians — including one family physician, one obstetrician–gynaecologist and

seven haematologists with expertise in VWD (two were paediatric haematologists). Ad hoc members of the Panel represented the Division of Blood Diseases and Resources of the NHLBI. The Panel was coordinated by the Office of Prevention, Education, and Control of the NHLBI. Panel members disclosed, verbally and in writing, any financial conflicts.

Barbara M. Alving MD, then acting director of the NHLBI, gave the charge to the Expert Panel to examine the current science in the area of VWD and to come to consensus regarding clinical recommendations for diagnosis, treatment and management of this common inherited bleeding disorder. The Panel was also charged to base each recommendation on current science and to indicate the strength of the relevant literature for each recommendation.

The development of this report was funded entirely by the NHLBI, National Institutes of Health (NIH). Panel members and reviewers participated as volunteers and were compensated only for travel expenses related to three in-person Expert Panel meetings.

After the Expert Panel finalized a basic outline for the guidelines, members were assigned to the three main sections: (i) Introduction and Background, (ii) Diagnosis and Evaluation, and (iii) Management of VWD. Three members were assigned lead responsibility for each section. The section groups were responsible for developing detailed outlines for the sections, reviewing the pertinent literature, writing the sections and drafting recommendations with the supporting evidence for the full Panel to review.

After internal NHLBI/NIH review of the draft document, it was posted on the NHLBI Web site for public review and comments. The draft was then revised by the Expert Panel in response to external review comments. The final document underwent NHLBI/NIH review before posting on the NHLBI Web site as well as finalization of a modified version for scientific journal publication.

Literature searches

Three section outlines, approved by the Expert Panel chair, were used as the basis for compiling relevant search terms, using the Medical Subject Headings (MeSH terms) of the MEDLINE database. If appropriate terms were not available in MeSH, then relevant non-MeSH key words were used. In addition to the search terms, inclusion and exclusion criteria were defined on the basis of feedback from the Panel about specific limits to include in the search strategies, specifically the following:

1 date restriction: 1990–2004;
2 language: English; and
3 study/publication types: randomized-controlled trial; meta-analysis; controlled clinical trial; epidemiological studies; prospective studies; multicentre study; clinical trial; evaluation studies; practice guideline; review, academic; review, multicase; technical report; validation studies; review of reported cases; case reports; journal article (to exclude letters, editorials, news, etc.).

The search strategies were constructed and executed in the MEDLINE database as well as in the Cochrane Database of Systematic Reviews to compile a set of citations and abstracts for each section. Initial searches on specific key word combinations and date and language limits were further refined by using the publication type limits to produce results that more closely matched the section outlines. Once the section results were compiled, the results were put in priority order by study type as follows:

1 randomized-controlled trial;
2 meta-analysis (quantitative summary combining results of independent studies);
3 controlled clinical trial;
4 multicentre study;
5 clinical trial (includes all types and phases of clinical trials);
6 evaluation studies;
7 practice guideline (for specific healthcare guidelines);
8 epidemiological studies;
9 prospective studies;
10 review, academic (comprehensive, critical, or analytical review);
11 review, multicase (review with epidemiological applications);
12 technical report;
13 validation studies;
14 review of reported cases (review of known cases of a disease); and
15 case reports.

On examination of the yield of the initial literature search, it was determined that important areas in the section outlines were not addressed by the citations, possibly because of the date delimiters. In addition, Panel members identified pertinent references from their own searches and databases, including landmark references predating the 1990 date restriction, and 2005 and 2006 references (to October 2006). Therefore, additional follow-up database searching was done using the same search strategies from the initial round, but covering dates before 1990 and during 2005 and 2006 to double-check for key studies appearing in the literature

outside the limits of the original range of dates. Also, refined searches in the 1990–2006 date range were conducted to analyse the references used by Panel members that had not appeared in the original search results. These revised searches helped round out the database search to provide the most comprehensive approach possible. As a result, the references used in the guidelines included those retrieved from the two literature searches combined with the references suggested by the Panel members. These references inform the guidelines and clinical recommendations, on the basis of the best available evidence in combination with the Panel's expertise and consensus.

Clinical recommendations – grading and levels of evidence

Recommendations made in this document are based on the levels of evidence described in Table 1, with a priority grading system of A, B or C. Grade A is reserved for recommendations based on evidence levels Ia and Ib. Grade B is given for recommendations having evidence levels of IIa, IIb and III. Grade C is for recommendations based on evidence level IV [8]. None of the recommendations merited a grade of A. Evidence tables are available on the NHLBI Web site for those recommendations graded as B with two or more references [10].

Table 1. Levels of evidence.

Level	Type of evidence
Ia	Evidence obtained from meta-analysis of randomized-controlled trials
Ib	Evidence obtained from at least one randomized-controlled trial
IIa	Evidence obtained from at least one well-designed controlled study without randomization
IIb	Evidence obtained from at least one other type of well-designed quasi-experimental study
III	Evidence obtained from well-designed non-experimental descriptive studies, such as comparative studies, correlation studies and case–control studies
IV	Evidence obtained from expert committee reports or opinions and/or clinical experiences of respected authorities

From Agency for Health Care Policy and Research. Acute pain management: operative or medical procedures and trauma. AHCPR Archived Clinical Practice Guidelines [Monograph on the Internet]. Rockville, MD: Agency for Health Care Policy and Research. Available at: http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=hstat6.chapter.8991

Scientific overview

Discovery and identification of VWD/VWF

The patient who led to the discovery of a hereditary bleeding disorder that we now call 'von Willebrand disease' was a 5-year-old girl who lived on the Åland Islands and was brought to Deaconess Hospital in Helsinki, Finland, in 1924 to be seen by Dr Erik von Willebrand [11]. He ultimately assessed 66 members of her family and reported in 1926 that this was a previously undescribed bleeding disorder that differed from haemophilia and exhibited (i) mucocutaneous bleeding, (ii) autosomal inheritance rather than being linked to the X chromosome, (iii) prolonged bleeding times (BT) by the Duke method (earlobe BT) and (iv) normal clotting time. Not only did he recognize the autosomal inheritance pattern, he also recognized that bleeding symptoms were greater in children and in women of child-bearing age. He subsequently found that blood transfusions were useful not only to correct the anaemia, but also to control bleeding.

In the 1950s, it became clear that a 'plasma factor', antihaemophilic factor (factor VIII [FVIII]), was decreased in these persons and that Cohn fraction I-0 could correct both the plasma deficiency of FVIII and the prolonged BT. For the first time, the factor causing the long BT was called 'von Willebrand factor'. As cryoprecipitate and commercial FVIII concentrates were developed, it was recognized that both VWF and antihaemophilic factor (FVIII) purified together.

When immunoassays were developed, persons who had VWD (in contrast to those who had haemophilia A) were found to have reduced factor VIII-related antigen (FVIIIR:Ag), which we now refer to as von Willebrand factor antigen (VWF:Ag). Characterization of the proteins revealed that FVIII was the clotting protein deficient in haemophilia A, and VWF was a separate FVIII carrier protein that resulted in the cofractionation of both proteins in commercial concentrates. Furthermore, a deficiency of VWF resulted in increased FVIII clearance because of the reduced carrier protein, VWF.

Since the 1980s, molecular and cellular studies have defined haemophilia A and VWD more precisely. Persons who had VWD had a normal FVIII gene on the X chromosome, but some were found to have an abnormal VWF gene on chromosome 12. Variant forms of VWF were recognized in the 1970s, and these variations are now recognized as the result of synthesis of an abnormal protein. Gene sequencing identified many of these persons as having a VWF

Table 2. Synopsis of VWF designations, properties and assays.

Designation	Property	Assay
VWF	Multimeric glycoprotein that promotes platelet adhesion and aggregation and is a carrier for FVIII in plasma	See specific VWF assays below
VWF:RCo	Binding activity of VWF that causes binding of VWF to platelets in the presence of ristocetin with consequent agglutination	Ristocetin cofactor activity: quantitates platelet agglutination after addition of ristocetin and VWF
VWF:Ag	VWF protein as measured by protein assays; does not imply functional ability	Immunological assays such as ELISA, LIA, RIA, Laurell electroimmunoassay
VWF:CB	Ability of VWF to bind to collagen	Collagen-binding activity: quantitates binding of VWF to collagen-coated ELISA plates
VWF multimers	Size distribution of VWF multimers as assessed by agarose gel electrophoresis	VWF multimer assay: electrophoresis in agarose gel and visualization by monospecific antibody to VWF
FVIII	Circulating coagulation protein that is protected from clearance by VWF and is important in thrombin generation	FVIII activity: plasma clotting test based on PTT assay using FVIII-deficient substrate; quantitates activity
RIPA	Test that measures the ability of a person's VWF to bind to platelets in the presence of various concentrations of ristocetin	RIPA: aggregation of a person's PRP to various concentrations of ristocetin

ELISA, enzyme-linked immunosorbent assay; FVIII, factor VIII; LIA, latex immunoassay (automated); PRP, platelet-rich plasma; PTT, activated partial thromboplastin time; RIA, radioimmunoassay; RIPA, ristocetin-induced platelet aggregation; VWF, von Willebrand factor; VWF:Ag, von Willebrand factor antigen; VWF:CB, von Willebrand factor collagen-binding activity; VWF:RCo, von Willebrand factor ristocetin cofactor activity.

gene mutation. The genetic causes of milder forms of low VWF are still under investigation, and these forms may not always be caused by an abnormal VWF gene. In addition, acquired disorders may result in reduced or dysfunctional VWF (discussed in a later section, Acquired von Willebrand Syndrome). Table 2 summarizes VWF designations, functions and assays. Table 3 lists and defines abbreviations used in the text of this document.

The VWF protein and its functions in vivo

VWF is synthesized in two cell types. In the vascular endothelium, VWF is synthesized and subsequently stored in secretory granules (Weibel-Palade bodies) from which it can be released by stress or drugs such as desmopressin (1-desamino-8-D-arginine, DDAVP; Sanofi-Aventis US, Bridgewater, NJ, USA), a synthetic analogue of vasopressin. VWF is also synthesized in bone marrow megakaryocytes where it is stored in platelet alpha-granules from which it is released after platelet activation. Desmopressin does not release platelet VWF.

VWF is a protein that is assembled from identical subunits into linear strings of varying size referred to as multimers. These multimers can be larger than 20 million daltons in mass and more than 2 μm long. The complex cellular processing consists of dimerization in the endoplasmic reticulum, glycosylation in the endoplasmic reticulum and Golgi complex, multimerization in the Golgi complex, and packaging into storage granules. The latter two processes are under the control of the VWF propeptide (VWFpp), which is cleaved from VWF at the time of storage. VWF that is released acutely into the circulation is accompanied by a parallel rise in FVIII, but it is still not entirely clear whether this protein–protein association first occurs within the endothelial cell [12,13].

In plasma, the FVIII–VWF complex circulates as a loosely coiled protein complex that does not interact strongly with platelets or endothelial cells under basal conditions. When vascular injury occurs, VWF becomes tethered to the exposed subendothelium (collagen, etc.). The high fluid shear rates that occur in the microcirculation appear to induce a conformational change in multimeric VWF that causes platelets to adhere, become activated, and then aggregate so as to present an activated platelet phospholipid surface. This facilitates clotting that is regulated in part by FVIII. Because of the specific characteristics of haemostasis and fibrinolysis on mucosal surfaces, symptoms in VWD are often greater in these tissues.

Plasma VWF is primarily derived from endothelial synthesis. Platelet and endothelial cell VWF are released locally after cellular activation where this VWF participates in the developing haemostatic plug or thrombus (Fig. 1).

Plasma VWF has a half-life of approximately 12 h (range: 9–15). VWF is present as very large multimers that are subjected to physiological degradation

Table 3. Nomenclature and abbreviations*.

Designation	Definition
ADAMTS13	A Disintegrin-like And Metalloprotease domain (reprolysin type) with ThromboSpondin type 1 motif, member 13: a plasma metalloprotease that cleaves multimeric VWF
AVWS	Acquired von Willebrand (disease) syndrome
BT	Bleeding time
CBC	Complete blood cell count
CV	Coefficient of variation
D&C	Dilation and curettage
DDAVP	1-Desamino-8-D-arginine; desmopressin
DVT	Deep vein thrombosis
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
FVIII[†]	[Blood clotting] factor VIII
FVIIIR:Ag[†]	Factor VIII-related antigen (see VWF:Ag)
GPIb	Glycoprotein Ib (platelet)
IGIV	Immune globulin intravenous (also known as IVIG)
ISTH	International Society on Thrombosis and Haemostasis
IU	International Unit
i.v.	intravenous(ly)
LIA	Latex immunoassay (automated)
MeSH	Medical Subject Headings (in MEDLINE)
MGUS	Monoclonal gammopathy of uncertain significance
NHLBI	National Heart, Lung, and Blood Institute
NIH	National Institutes of Health
NSAID	Non-steroidal anti-inflammatory drug
PCR	Polymerase chain reaction
PFA-100	Platelet function analyzer
PLT-VWD	Platelet-type von Willebrand disease
PTT	Partial thromboplastin time (activated partial thromboplastin time)
RIPA	Ristocetin-induced platelet aggregation
s.c.	subcutaneous(ly)
TTP	Thrombotic thrombocytopenic purpura
tPA	Tissue plasminogen activator
VWD	von Willebrand disease
VWF[†]	von Willebrand factor (FVIII carrier protein)
VWF:Ag[†]	von Willebrand factor antigen
VWF:CB[†]	von Willebrand factor collagen-binding activity
VWF:FVIIIB[†]	von Willebrand factor: factor VIII-binding assay
VWF:PB assay	von Willebrand factor platelet-binding assay
VWFpp	von Willebrand factor propeptide
VWF:RCo[†]	von Willebrand factor ristocetin cofactor activity
WHO	World Health Organization

*Abbreviations listed appear in the text. Abbreviations in the tables are expanded in each table's footnotes.
[†]These abbreviations (for FVIII and VWF and all their properties) are defined in Marder *et al.* [397] and Mazurier and Rodeghiero [398].

by the metalloprotease ADAMTS13 [A Disintegrin-like And Metalloprotease domain (reprolysin type) with ThromboSpondin type 1 motif, member 13]. Deficiency of ADAMTS13 is associated with the pathological microangiopathy of thrombotic throm-

bocytopenic purpura (TTP). The most common variant forms of type 2A VWD are characterized by increased VWF susceptibility to ADAMTS13.

Factors affecting VWF levels Factors that affect levels of plasma VWF include age, race, ABO and Lewis blood groups, epinephrine, inflammatory mediators, and endocrine hormones (particularly those associated with the menstrual cycle and pregnancy). VWF is increased during pregnancy (a three- to fivefold elevation over a woman's baseline by the third trimester) with ageing and with acute stress or inflammation. Africans and African-Americans have higher average levels of VWF than the White or caucasian populations [14,15]. VWF is reduced by hypothyroidism and rarely by autoantibodies to VWF. The rate of VWF synthesis probably is not affected by blood group; however, the survival of VWF appears to be reduced in individuals who have type O blood. In fact, ABO blood group substance has been identified on VWF.

The genetics of VWD

Since the 1980s, molecular and cellular studies have defined haemophilia A and VWD more precisely. Persons with severe VWD have a normal FVIII gene on the X chromosome, and some are found to have an abnormal VWF gene on chromosome 12. The VWF gene is located near the tip of the short arm of chromosome 12, at 12p13.3 [16]. It spans approximately 178 kb of DNA and contains 52 exons [17]. Intron–exon boundaries tend to delimit structural domains in the protein, and introns often occur at similar positions within the gene segments that encode homologous domains. Thus, the structure of the VWF gene reflects the mosaic nature of the protein (Fig. 2).

A partial, unprocessed VWF pseudogene is located at chromosome 22q11.2 [18]. This pseudogene spans approximately 25 kb of DNA and corresponds to exons 23–34 and part of the adjacent introns of the VWF gene [19]. This segment of the gene encodes domains A1A2A3, which contain binding sites for platelet glycoprotein Ib (GPIb) and collagen, as well as the site cleaved by ADAMTS13. The VWF pseudogene and gene have diverged 3.1% in DNA sequence, consistent with a relatively recent origin of the pseudogene by partial gene duplication [19]. This pseudogene is found in humans and great apes (bonobo, chimpanzee, gorilla and orangutan) but not in more distantly related primates [20]. The VWF pseudogene complicates the detection of VWF gene mutations because polymerase chain reactions
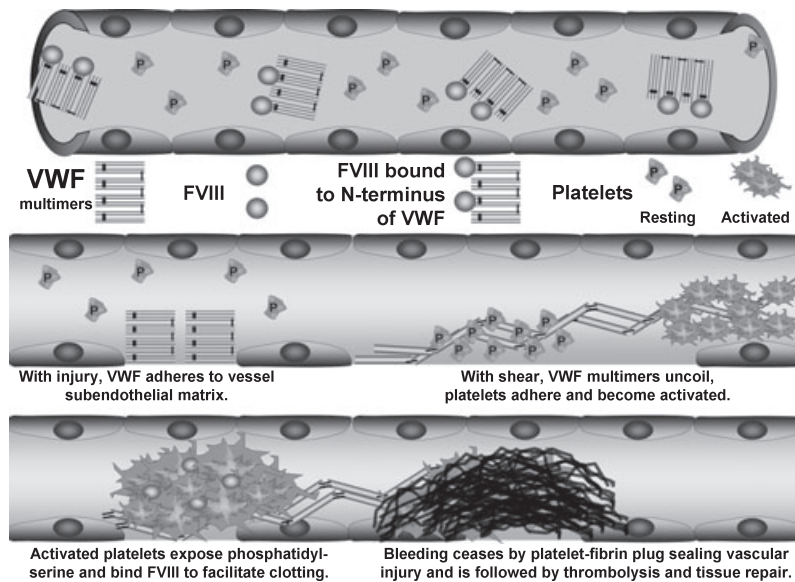
Fig. 1. von Willebrand factor (VWF) and normal haemostasis. A blood vessel cross-section shows stages of normal haemostasis. Top, VWF is the carrier protein for blood clotting factor VIII (FVIII). Under normal conditions VWF does not interact with platelets or the blood vessel wall that is covered with endothelial cells. Middle left, After vascular injury, VWF adheres to the exposed subendothelial matrix. Middle right, After VWF is uncoiled by local shear forces, platelets adhere to the altered VWF, and these platelets undergo activation and recruit other platelets to this injury site. Bottom left, The activated and aggregated platelets alter their membrane phospholipids exposing phosphatidylserine, and this activated platelet surface binds clotting factors from circulating blood and initiates blood clotting on this surface where fibrin is locally deposited. Bottom right, The combination of clotting and platelet aggregation and adhesion forms a platelet-fibrin plug, which results in the cessation of bleeding. The extent of the clotting is carefully regulated by natural anticoagulants. Subsequently, thrombolysis initiates tissue repair, and ultimately the vessel may be re-endothelialized and blood flow maintained. (Courtesy of R. R. Montgomery, the BloodCenter of Wisconsin and Medical College of Wisconsin, Milwaukee, Wisconsin; used with permission).
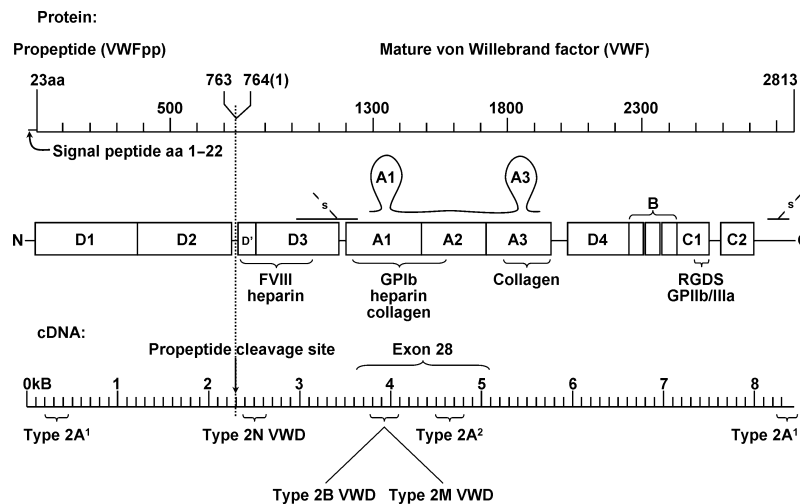

Fig. 2. Structure and domains of von Willebrand factor (VWF). The VWF protein sequence [amino acid (aa) 1–2813] is aligned with the cDNA sequence (nucleic acid 1–8439). The VWF signal peptide is the first 22 aa, the propeptide (VWFpp) is aa 23–763 and mature VWF is aa 764–2800. Type 2 mutations are primarily located in specific domains (regions) along the VWF protein. Types 2A, 2B and 2M VWF mutations are primarily located within exon 28 that encodes for the A1 and A2 domains of VWF. The two different types of 2A are those that have increased proteolysis ($2A^2$) and those with abnormal multimer synthesis ($2A^1$). Type 2N mutations are located within the D′ and D3 domains. Ligands that bind to certain VWF domains are identified, including factor VIII (FVIII), heparin, platelet glycoprotein Ib complex (GPIb), collagen and platelet glycoprotein IIb/IIIa complex (GPIIb/IIIa) that binds to the RGD (arginine-glycine-aspartate) amino acid sequence in VWF. (Courtesy of R. R. Montgomery, the BloodCenter of Wisconsin and Medical College of Wisconsin, Milwaukee, Wisconsin; used with permission.)

(PCRs) can inadvertently amplify segments from either or both loci, but this difficulty can be overcome by careful design of gene-specific PCR primers [19].

The VWF pseudogene may occasionally serve as a reservoir of mutations that can be introduced into the VWF locus. For example, some silent and some potentially pathogenic mutations have been identified in exons 27 and 28 of the VWF gene of persons who have VWD. These same sequence variations occur consecutively in the VWF pseudogene and might have been transferred to the VWF by gene conversion [21–23]. The segments involved in the potential gene conversion events are relatively short, from a minimum of seven nucleotides [21] to a maximum of 385 nucleotides [23]. The frequency of these potential interchromosomal exchanges is unknown.

The spectrum of VWF gene mutations that cause VWD is similar to that of many other human genetic diseases and includes large deletions, frameshifts from small insertions or deletions, splice-site mutations, nonsense mutations causing premature termination of translation, and missense mutations affecting single amino acid residues. A database of VWF mutations and polymorphisms has been compiled for the International Society on Thrombosis and Haemostasis (ISTH) [24,25] and is maintained for online access at the University of Sheffield [26]. Mutations causing VWD have been identified throughout the VWF gene. In contrast to haemophilia A, in which a single major gene rearrangement causes a large fraction of severe disease, no such recurring mutation is common in VWD. There is a good correlation between the location of mutations in the VWF gene and the subtype of VWD, as discussed in more detail in the next section, Classification of VWD Subtypes. In selected families, this information can facilitate the search for VWF mutations by DNA sequencing.

Classification of VWD subtypes

VWD is classified on the basis of criteria developed by the VWF Subcommittee of the ISTH, first published in 1994 and revised in 2006 (Table 4) [27,28].

The classification was intended to be clinically relevant to the treatment of VWD. Diagnostic categories were defined that encompassed distinct pathophysiological mechanisms and correlated with the response to treatment with desmopressin or blood products. The classification was designed to be conceptually independent of specific laboratory

Table 4. Classification of von Willebrand disease*.

Type	Description
1	Partial quantitative deficiency of VWF
2	Qualitative VWF defect
2A	Decreased VWF-dependent platelet adhesion with selective deficiency of high-molecular-weight multimers
2B	Increased affinity for platelet GPIb
2M	Decreased VWF-dependent platelet adhesion without selective deficiency of high-molecular-weight multimers
2N	Markedly decreased binding affinity for FVIII
3	Virtually complete deficiency of VWF

FVIII, factor VIII; GPIb, glycoprotein Ib (platelet); VWF, von Willebrand factor.
*Disease types are defined as described in Sadler *et al.* [27].

testing procedures, although most of the VWD subtypes could be assigned by using tests that were widely available. The 1994 classification reserved the designation of VWD for disorders caused by mutations within the VWF gene [28], but this criterion has been dropped from the 2006 classification [27] because, in practice, it is verifiable for only a small fraction of patients.

VWD is classified into three major categories: partial quantitative deficiency (type 1), qualitative deficiency (type 2) and total deficiency (type 3). Type 2 VWD is divided further into four variants (2A, 2B, 2M and 2N) on the basis of details of the phenotype. Before publication of the 1994 revised classification of VWD [28], VWD subtypes were classified using roman numerals (types I, II, and III), generally corresponding to types 1, 2 and 3 in the 1994 classification, and within type II several subtypes existed (designated by adding sequential letters of the alphabet, i.e. II-A through II-I). Most of the latter VWD variants were amalgamated as type 2A in the 1994 classification, with the exception of type 2B (formerly II-B), for which a separate new classification was created. In addition, a new subtype (2M, with 'M' representing 'multimer') was created to include variants with decreased platelet-dependent function [VWF ristocetin cofactor activity (VWF:RCo)] but no significant decrease of higher molecular weight VWF multimers (which may or may not have other aberrant structure). Subtype 2N VWD was defined, with 'N' representing 'Normandy', where the first individuals were identified, with decreased FVIII because of VWF defects of FVIII binding.

Type 1 VWD affects approximately 75% of symptomatic persons who have VWD (see Castaman *et al.* [29] for a review). Almost all the remaining persons are divided among the four type 2 variants,

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Table 5. Inheritance, prevalence and bleeding propensity in patients with von Willebrand disease.

Type	Inheritance	Prevalence	Bleeding propensity
1	Autosomal dominant	Up to 1%	Mild to moderate
2A	Autosomal dominant (or recessive)	Uncommon	Variable, usually moderate
2B	Autosomal dominant	Uncommon	Variable, usually moderate
2M	Autosomal dominant (or recessive)	Uncommon	Variable, usually moderate
2N	Autosomal recessive	Uncommon	Variable, usually moderate
3 (severe)	Autosomal recessive	Rare (1:250 000–1:1 000 000)	High (severe bleeding)

and the partitioning among them varies considerably among centres. In France, for example, patients' distribution has been reported to be 30% type 2A, 28% type 2B, 8% type 2M (or unclassified) and 34% type 2N [30]. In Bonn, Germany, the distribution has been reported to be 74% type 2A, 10% type 2B, 13% type 2M and 3.5% type 2N [31]. Table 5 summarizes information about inheritance, prevalence and bleeding propensity in persons who have different types of VWD.

The prevalence of type 3 VWD in the population is not known precisely but has been estimated (per million population) as 0.55 for Italy [32], 1.38 for North America [33], 3.12 for Sweden [32] and 3.2 for Israel [34]. The prevalence may be as high as 6 per million where consanguinity is common [1].

Type 1 VWD Type 1 VWD is found in persons who have partial quantitative deficiency of VWF. The level of VWF in plasma is low, and the remaining VWF mediates platelet adhesion normally and binds FVIII normally. Laboratory evaluation shows concordant decreases in VWF protein concentration (VWF:Ag) and assays of VWF function (VWF:RCo). Levels of blood clotting FVIII usually parallel VWF and may be reduced secondary to reduced VWF. Usually, in type 1 VWD, the FVIII/VWF:Ag ratio is 1.5–2.0. In most persons with type 1 VWD, this results in normal or mildly decreased FVIII, not reduced as much as the VWF. VWF multimer gels show no significant decrease in large VWF multimers [28]. The laboratory evaluation of VWD is discussed in the Diagnosis and Evaluation section.

The spectrum of mutations occurring in VWD type 1 has been described extensively in two major studies [35,36]. Particularly severe, highly penetrant forms of type 1 VWD may be caused by dominant VWF mutations that interfere with the intracellular transport of dimeric proVWF [37–41] or that promote the rapid clearance of VWF from the circulation [40,42,43]. Persons who have such mutations usually have plasma VWF levels <20 IU dL^{-1} (International Units per deciliter) [35,36]. Most of the mutations characterized to date cause single amino acid substitutions in domain D3 [37–39,41,44]. One mutation

associated with rapid clearance has been reported in domain D4 [40].

Increased clearance of VWF from the circulation in type 1 VWD may account for the exaggerated but unexpectedly brief responses to desmopressin observed in some patients. Consequently, better data on the prevalence of increased clearance could affect the approach to diagnosing type 1 VWD and the choice of treatment for bleeding.

A diagnosis of type 1 VWD is harder to establish when the VWF level is not markedly low but instead is near the lower end of the normal range. Type 1 VWD lacks a qualitative criterion by which it can be recognized and instead relies only on quantitative decrements of protein concentration and function. VWF levels in the healthy population span a wide range of values. The mean level of plasma VWF is 100 IU dL^{-1}, and approximately 95% of plasma VWF levels lie between 50 and 200 IU dL^{-1} [45,46]. Because mild bleeding symptoms are common in the healthy population, the association of bleeding symptoms with a moderately low VWF level may be coincidental [47]. The conceptual and practical issues associated with the evaluation of moderately low VWF levels are discussed more completely later in this section. (See section Type 1 VWD vs. Low VWF: VWF Level as a Risk Factor for Bleeding.)

Type 2 VWD The clinical features of several type 2 VWD variants are distinct from those of type 1 VWD, and they can have strikingly distinct and specific therapeutic needs. As a consequence, the medical care of patients with type 2 VWD benefits from the participation of a haematologist who has expertise in haemostasis. Bleeding symptoms in type 2 VWD are often thought to be more severe than those in type 1 VWD, although this impression needs to be evaluated in suitable clinical studies.

Type 2A VWD. Type 2A VWD refers to qualitative variants in which VWF-dependent platelet adhesion is decreased because the proportion of large VWF multimers is decreased. Levels of VWF:Ag and FVIII may be normal or modestly decreased, but VWF function is abnormal, as shown by markedly decreased VWF:RCo [48]. Type 2A

VWD may be caused by mutations that interfere with the assembly or secretion of large multimers or by mutations that increase the susceptibility of VWF multimers to proteolytic degradation in the circulation [49–51]. The deficit of large multimers predisposes persons to bleed.

The location of type 2A VWD mutations sometimes can be inferred from high-resolution VWF multimer gels. For example, mutations that primarily reduce multimer assembly lead to the secretion of multimers that are too small to engage platelets effectively and therefore are relatively resistant to proteolysis by ADAMTS13. Homozygous mutations in the propeptide impair multimer assembly in the Golgi complex and give rise to a characteristic 'clean' pattern of small multimers that lack the satellite bands usually associated with proteolysis (see Diagnosis and Evaluation section); this pattern was initially described as 'type IIC' VWD [52–54]. Heterozygous mutations in the cystine knot domain can impair dimerization of proVWF in the endoplasmic reticulum and cause a recognizable multimer pattern originally referred to as 'type IID' [55,56]. A mixture of monomers and dimers arrives in the Golgi complex, where the incorporation of monomers at the end of a multimer prevents further elongation. As a result, the secreted small multimers contain minor species with an odd number of subunits that appear as faint bands between the usual species that contain an even number of subunits. Heterozygous mutations in cysteine residues of the D3 domain can also impair multimer assembly, but these mutations often also produce an indistinct or 'smeary' multimer pattern referred to as 'type IIE' [57,58].

In contrast to mutations that primarily affect multimer assembly, mutations within or near the A2 domain of VWF cause type 2A VWD, which is associated with markedly increased proteolysis of the VWF subunits [58] (Fig. 2). These mutations apparently interfere with the folding of the A2 domain and make the Tyr1605–Met1606 bond accessible to ADAMTS13 even in the absence of increased fluid shear stress. Two subgroups of this pattern have been distinguished: group I mutations enhance proteolysis by ADAMTS13 and also impair multimer assembly, whereas group II mutations enhance proteolysis without decreasing the assembly of large VWF multimers [51]. Computer modelling of domain A2 suggests that group I mutations affect both assembly and proteolysis, because group I mutations have a more disruptive effect on the folding of domain A2 than do group II mutations [59].

Type 2B VWD. Type 2B VWD is caused by mutations that pathologically increase platelet-VWF binding, which leads to the proteolytic degradation and depletion of large, functional VWF multimers [58,60]. Circulating platelets are also coated with mutant VWF, which may prevent the platelets from adhering at sites of injury [61].

Although laboratory results for type 2B VWD may be similar to those in type 2A or type 2M VWD, patients with type 2B VWD typically have thrombocytopenia that is exacerbated by surgery, pregnancy or other stress [62–64]. The thrombocytopenia probably is caused by reversible sequestration of VWF-platelet aggregates in the microcirculation. These aggregates are dissolved by the action of ADAMTS13 on VWF, causing the characteristic decrease of large VWF multimers and the prominent satellite banding pattern that indicates increased proteolytic degradation [65,66]. The diagnosis of type 2B VWD depends on finding abnormally increased ristocetin-induced platelet aggregation (RIPA) at low concentrations of ristocetin.

Type 2B VWD mutations occur within or adjacent to VWF domain A1 [24,57,67–70], which changes conformation when it binds to platelet GPIb [71]. The mutations appear to enhance platelet binding by stabilizing the bound conformation of domain A1.

Type 2M VWD. Type 2M VWD includes variants with decreased VWF-dependent platelet adhesion that is not caused by the absence of high-molecular-weight VWF multimers. Instead, type 2M VWD mutations reduce the interaction of VWF with platelet GPIb or with connective tissue and do not substantially impair multimer assembly. Screening laboratory results in type 2M VWD and type 2A VWD are similar, and the distinction between them depends on multimer gel electrophoresis [69].

Mutations in type 2M VWD have been identified in domain A1 (Fig. 2), where they interfere with binding to platelet GPIb [24,57,69,72–74]. One family has been reported in which a mutation in VWF domain A3 reduces VWF binding to collagen, thereby reducing platelet adhesion and possibly causing type 2M VWD [75].

Type 2N VWD. Type 2N VWD is caused by VWF mutations that impair binding to FVIII, lowering FVIII levels so that type 2N VWD masquerades as an autosomal recessive form of haemophilia A [76–78]. In typical cases, the FVIII level is <10% (IU dL^{-1}), with a normal VWF:Ag and VWF:RCo. Discrimination from haemophilia A may require assays of FVIII-VWF binding [79,80].

Most mutations that cause type 2N VWD occur within the FVIII binding site of VWF (Fig. 2), which lies between residues Ser764 and Arg1035 and spans

domain D′ and part of domain D3 [24,81,82]. The most common mutation, Arg854Gln, has a relatively mild effect on FVIII binding and tends to cause a less severe type 2N VWD phenotype [79]. Some mutations in the D3 domain C-terminal of Arg1035 can reduce FVIII binding [83–85], presumably through an indirect effect on the structure or accessibility of the binding site.

Type 3 VWD Type 3 VWD is characterized by undetectable VWF protein and activity, and FVIII levels usually are very low (1–9 IU dL^{-1}) [86–88]. Nonsense and frameshift mutations commonly cause type 3 VWD, although large deletions, splice-site mutations and missense mutations can also do so. Mutations are distributed throughout the VWF gene, and most are unique to the family in which they were first identified [24,89,90].

A small fraction of patients with type 3 VWD develop alloantibodies to VWF in response to the transfusion of plasma products. These antibodies have been reported in 2.6–9.5% of patients with type 3 VWD, as determined by physician surveys or screening [87,91]. The true incidence is uncertain, however, because of unavoidable selection bias in these studies. Anti-VWF alloantibodies can inhibit the haemostatic effect of blood product therapy and also may cause life-threatening allergic reactions [87,92]. Large deletions in the VWF gene may predispose patients to this complication [91].

VWD classification, general issues The principal difficulties in using the current VWD classification concern how to define the boundaries between the various subtypes through laboratory testing. In addition, some mutations have pleiotropic effects on VWF structure and function, and some persons are compound heterozygous for mutations that cause VWD by different mechanisms. This heterogeneity can produce complex phenotypes that are difficult to categorize. Clinical studies of the relationship between VWD genotype and clinical phenotype would be helpful to improve the management of patients with the different subtypes of VWD.

The distinction between quantitative (type 1) and qualitative (type 2) defects depends on the ability to recognize discrepancies among VWF assay results [82,93], as discussed in the section Diagnosis and Evaluation. Similarly, distinguishing between type 2A and type 2M VWD requires multimer gel analysis. Standards need to be established for using laboratory tests to make these important distinctions.

The example of Vicenza VWD illustrates some of these problems. Vicenza VWD was first described as a variant of VWD in which the level of plasma VWF is usually <15 IU dL^{-1} and the VWF multimers are even larger than normal, like the ultralarge multimers characteristic of platelet VWF [94]. The low level of VWF in plasma in Vicenza VWD appears to be explained by the effect of a specific mutation, Arg1205His, that promotes clearance of VWF from the circulation about five times more rapidly than normal [43]. Because the newly synthesized multimers have less opportunity to be cleaved by ADAMTS13 before they are cleared, accelerated clearance alone may account for the increased multimer size in Vicenza VWD [95]. Whether Vicenza VWD is classified under type 1 VWD or type 2M VWD depends on the interpretation of laboratory test results. The abnormally large multimers and low RIPA values have led some investigators to prefer the designation of type 2M VWD [96]. However, the VWF:RCo/VWF:Ag ratio typically is normal, and large VWF multimers are not decreased relative to smaller multimers, so that other investigators have classified Vicenza VWD under type 1 VWD [43]. Regardless of how this variant is classified, the markedly shortened half-life of plasma VWF in Vicenza VWD is a key fact that, depending on the clinical circumstance, may dictate whether the patient should receive treatment with desmopressin or FVIII/VWF concentrates.

Type 1 VWD vs. low VWF: VWF level as a risk factor for bleeding

Persons who have very low VWF levels, i.e. <20 IU dL^{-1}, are likely to have VWF gene mutations, significant bleeding symptoms and a strongly positive family history [35,36,39,97–101]. Diagnosing type 1 VWD in these persons seems appropriate because they may benefit from changes in lifestyle and from specific treatments to prevent or control bleeding. Identification of affected family members may also be useful, and genetic counselling is simplified when the pattern of inheritance is straightforward.

In contrast, persons with VWF levels of 30–50 IU dL^{-1}, just below the usual normal range (50–200 IU dL^{-1}), pose problems for diagnosis and treatment. Among the total US population of approximately 300 million, VWF levels <50 IU dL^{-1} are expected in about 7.5 million persons, who therefore would be at risk for a diagnosis of type 1 VWD. Because of the strong influence of ABO blood group on VWF level [45], about 80% of US residents

who have low VWF have also blood type O. Furthermore, moderately low VWF levels and bleeding symptoms generally are not coinherited within families and are not strongly associated with intragenic VWF mutations [102–104]. In a recent Canadian study of 155 families who had type 1 VWD, the proportion showing linkage to the VWF locus was just 41% [100]. In a similar European study, linkage to the VWF locus depended on the severity of the phenotype. If plasma levels of VWF were <30 IU dL^{-1}, linkage was consistently observed, but if levels of VWF were higher than 30 IU dL^{-1}, the proportion of linkage was only 51% [99]. Furthermore, bleeding symptoms were not significantly linked to the VWF gene in these families [99].

Family studies suggest that 25–32% of the variance in plasma VWF is heritable [105,106]. Twin studies have reported greater heritability of 66–75% [107,108], although these values may be overestimates because of shared environmental factors [106,109]. Therefore, it appears that, at least in the healthy population, a substantial fraction of the variation in VWF level is not heritable.

Few genes have been identified that contribute to the limited heritability of VWF level. The major genetic influence on VWF level is the ABO blood group, which is thought to account for 20–30% of its heritable variance [14,108,110]. The mean VWF level for blood type O is 75 U dL^{-1}, which is 25–35 U dL^{-1} lower than other ABO types, and 95% of VWF levels for type O blood donors are between 36 and 157 U dL^{-1} [45]. The *Secretor* locus has a smaller effect. *Secretor*-null persons have VWF levels

slightly lower than those who have the *Secretor* locus [111]. An effect of the VWF locus has been difficult to discern by linkage analysis. One study suggested that 20% of the variance in VWF levels is attributable to the VWF gene [110], whereas another study could not demonstrate such a relationship [112].

In sum, known genetic factors account for a minority of the heritable variation in VWF level, and moderately low VWF levels (30–50 IU dL^{-1}) do not show consistent linkage to the VWF locus [99,100,102,103]. The diagnosis and management of VWD would be facilitated by better knowledge of how inherited and environmental factors influence the plasma concentration of VWF.

The attribution of bleeding to a low VWF level can be difficult because mild bleeding symptoms are very common, as discussed in the section Diagnosis and Evaluation, and the risk of bleeding is only modestly increased for persons who have moderately decreased VWF levels [47]. For example, in the course of investigating patients with type 3 VWD, approximately 190 obligate heterozygous relatives have had bleeding histories obtained and VWF levels measured (Table 6). The geometric mean VWF level was 47 IU dL^{-1} [47], with a range (±2 SD) of 16–140 IU dL^{-1}. Among 117 persons who had VWF <50 IU dL^{-1}, 31 (26%) had bleeding symptoms. Among 74 persons who had VWF higher than 50 IU dL^{-1}, 10 (14%) had bleeding symptoms. Therefore, the relative risk of bleeding was 1.9 ($P = 0.046$, Fisher's exact test) for persons who had low VWF. There was a trend for an increased frequency of bleeding symptoms at the lowest VWF

Table 6. Bleeding and VWF level in type 3 VWD heterozygotes.

Source	Setting	Population	Results
Castaman *et al.* [399]	1 family with type 3 proband	11 heterozygous	None with bleeding; 6 with VWF <50 IU dL^{-1}
Eikenboom *et al.* [22]	8 families with type 3 probands	22 heterozygous	2 who had mild bleeding among 9 with VWF <50 IU dL^{-1}
Zhang *et al.* [400]	13 families with type 3 probands	55 heterozygous	22 who had mild bleeding among 38 with VWF <50 IU dL^{-1}; 9 who had mild bleeding among 17 with VWF >50 IU dL^{-1}
Schneppenheim *et al.* [113]	22 families with type 3 probands	44 heterozygous	5 who had epistaxis, bruising or menorrhagia among 24 with VWF <50 IU dL^{-1}; 1 who had postoperative bleeding among 20 with VWF >50 IU dL^{-1}
Eikenboom *et al.* [199]	1 family with type 3 proband	4 heterozygous	2 who had mild bleeding among 4 with VWF <50 IU dL^{-1}
Inbal *et al.* [401]	4 families with type 3 probands	20 heterozygous	None who had bleeding; 15 with VWF <50 IU dL^{-1}
Nichols *et al.* [402]	1 family with type 3 proband	6 heterozygous	None with bleeding; 2 with VWF <50 IU dL^{-1}
Mannucci *et al.* [46]	15 families with type 3 probands	28 heterozygous	None who had bleeding; 19 with VWF <50 IU dL^{-1}

VWD, von Willebrand disease; VWF, von Willebrand factor.

levels: among 31 persons who had VWF levels <30 IU dL^{-1}, 12 (39%) had symptoms. Bleeding was mild and consisted of epistaxis, bruising, menorrhagia and bleeding after tooth extraction. The one person who experienced postoperative bleeding had a VWF level higher than 50 IU dL^{-1} [113].

The management of bleeding associated with VWF deficiency would be facilitated by better understanding of the heritability of low VWF levels (in the range of 20–50 IU dL^{-1}), their association with intragenic VWF mutations, and their interactions with other modifiers of bleeding risk. Such data could provide a foundation for treating VWF level as a biomarker for a moderate risk of bleeding, much as high blood pressure and high cholesterol are treated as biomarkers for cardiovascular disease risk.

Acquired von Willebrand syndrome

Acquired von Willebrand syndrome (AVWS) refers to defects in VWF concentration, structure or function that are not inherited directly but are consequences of other medical disorders. Laboratory findings in AVWS are similar to those in VWD and may include decreased values for VWF:Ag, VWF:RCo or FVIII. The VWF multimer distribution may be normal, but the distribution often shows a decrease in large multimers similar to that seen in type 2A VWD [114,115]. AVWS usually is caused by one of three mechanisms: autoimmune clearance or inhibition of VWF, increased shear-induced proteolysis of VWF, or increased binding of VWF to platelets or other cell surfaces. Autoimmune mechanisms may cause AVWS in association with lymphoproliferative diseases, monoclonal gammopathies, systemic lupus erythematosus, other autoimmune disorders and some cancers. Autoantibodies to VWF have been detected in <20% of patients in whom they have been sought, suggesting that the methods for antibody detection may not be sufficiently sensitive or that AVWS in these settings may not always have an autoimmune basis.

Pathological increases in fluid shear stress can occur with cardiovascular lesions, such as ventricular septal defect and aortic stenosis, or with primary pulmonary hypertension. The increased shear stress can increase the proteolysis of VWF by ADAMTS13 enough to deplete large VWF multimers and thereby produce a bleeding diathesis that resembles type 2A VWD. The VWF multimer distribution improves if the underlying cardiovascular condition is treated successfully [114–119].

Increased binding to cell surfaces, particularly platelets, can also consume large VWF multimers.

An inverse relationship exists between the platelet count and VWF multimer size, probably because increased encounters with platelets promote increased cleavage of VWF by ADAMTS13. This mechanism probably accounts for AVWS associated with myeloproliferative disorders; reduction of the platelet count can restore a normal VWF multimer distribution [120–122]. In rare instances, VWF has been reported to bind GPIb that was expressed ectopically on tumour cells [115,123].

Acquired von Willebrand syndrome has been described in hypothyroidism caused by non-immune mechanism [124]. Several drugs have been associated with AVWS; those most commonly reported include valproic acid, ciprofloxacin, griseofulvin and hydroxyethyl starch [114,115].

Acquired von Willebrand syndrome occurs in various conditions, but other clinical features may direct attention away from this potential cause of bleeding. More studies are needed to determine the incidence of AVWS and to define its contribution to bleeding in the many diseases and conditions with which it is associated.

Prothrombotic clinical issues and VWF in persons who do not have VWD

Whether elevation of VWF is prothrombotic has been the subject of several investigations. Both arterial and venous thrombotic disorders have been studied.

Open heart surgery Haemostatic activation after open heart surgery has been suggested as a mechanism of increased risk of postoperative thrombosis in this setting. A randomized trial comparing coronary artery surgery with or without cardiopulmonary bypass ('off-pump') found a consistent and equivalent rise in VWF:Ag levels at 1–4 postoperative days in the two groups [125], suggesting that the surgery itself, rather than cardiopulmonary bypass, was responsible for the rise in VWF. There is no direct evidence that the postoperative rise in VWF contributes to the risk of thrombosis after cardiac surgery.

Coronary artery disease Three large prospective studies of subjects without evidence of ischaemic heart disease at entry have shown, by univariate analysis, a significant association of VWF:Ag level at entry with subsequent ischaemic coronary events [126–128]. However, the association remained significant by multivariate analysis in only one subset of subjects in these studies [126], a finding that could

have occurred by chance. These findings suggest that the association of VWF with the incidence of coronary ischaemic events is relatively weak and may not be directly causal.

Thrombosis associated with atrial fibrillation A prospective study of vascular events in subjects with atrial fibrillation found, by univariate analysis, a significant association of VWF:Ag level with subsequent stroke or vascular events. The association with vascular events remained significant with multivariate analysis [129].

Thrombotic thrombocytopenic purpura The hereditary deficiency or acquired inhibition of a VWF-cleaving protease, ADAMTS13, is associated with the survival in plasma of ultralarge VWF multimers, which are involved in the propensity to development of platelet-rich thrombi in the microvasculature of individuals who have TTP [130,131].

Deep vein thrombosis In a case–control study of 301 patients, evaluated at least 3 months after cessation of anticoagulation treatment for a first episode of deep vein thrombosis (DVT), plasma levels of VWF:Ag and FVIII activity were related to risk of DVT, according to univariate analysis. In multivariate analysis, the relation of VWF level with risk of DVT was not significant after adjustment for FVIII levels [132].

Diagnosis and evaluation

Introduction

The evaluation of a person for possible VWD or other bleeding disorders may be initiated because of various clinical indications (Fig. 3). These indications and situations may include evaluation of (i) an asymptomatic person who will undergo a surgical or interventional procedure; (ii) persons who present with current symptoms of or a history of increased bleeding, abnormal laboratory studies, a positive family history of a bleeding disorder or a combination of these factors; or (iii) persons who present with a prior diagnosis of VWD but do not have supporting laboratory documentation. In all cases, the initial step in assessment should focus on key aspects of the person's clinical history to determine whether the person may benefit from further diagnostic evaluation. This section is divided into two parts. The first part uses a summary of the medical literature to suggest questions for an initial assessment of persons presenting with concerns about bleeding issues or for evaluation before procedures that may increase their risk of bleeding. Using the answers to the initial assessment, the second part focuses on a strategy for optimal laboratory assessment of persons who potentially have bleeding disorders and suggests guidelines for interpretation of laboratory results.

Evaluation of the patient

History, signs and symptoms The initial clinical assessment of a person who is being evaluated for VWD should focus on a personal history of excessive bleeding throughout the person's life and any family history of a bleeding disorder. The history of bleeding should identify the spontaneity and severity, sites of bleeding, duration of bleeding, type of insult or injury associated with bleeding, ease with which bleeding can be stopped and concurrent medications — such as aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs), clopidogrel (Plavix), warfarin or heparin — at the onset of bleeding. Particularly when an invasive procedure is anticipated, the person should be asked whether he or she is currently taking any of these medications and also whether he or she has any history of liver or kidney disease, blood or bone marrow disease or high or low-platelet counts. If a history of any of these disorders is present, further appropriate evaluation or referral should be undertaken.

Clinical manifestations The most common presenting symptoms in persons who receive a diagnosis of VWD are summarized in Table 7. Symptoms usually involve mucous membranes and skin sites, and bleeding is of mild-to-moderate severity (bleeding that does not require blood transfusions and usually does not require visits to the physician) for most persons with VWD, reflecting the predominance of type 1 VWD. However, life-threatening bleeding (central nervous system and gastrointestinal tract) can occur in persons with type 3 VWD, in some persons with type 2 VWD, and rarely in persons with type 1 VWD. Uncommon bleeding manifestations, such as haemarthrosis, are more common in persons who have a more severe deficiency, especially those with type 3 VWD [87,133]. Clinical symptoms may also be modified by coexisting illnesses or other medications. For example, use of aspirin or NSAIDs can exacerbate the bleeding tendency, whereas use of oral contraceptives can decrease bleeding in women with VWD.

The clinical evaluation of bleeding symptoms is a challenge, because mild bleeding symptoms are also

Questions to Patient

(for example, an asymptomatic person who will undergo a surgical or other invasive procedure)

1. Have you or a blood relative ever needed medical attention for a bleeding problem or been told you have a bleeding disorder or problem?
   • During or after surgery?
   • With dental procedures or extractions?
   • With trauma?
   • During childbirth or for heavy menses?
   • Ever had bruises with lumps?
2. Do you have or have you ever had
   • Liver or kidney disease; a blood or bone marrow disorder; a high or low platelet count?
3. Do you take aspirin, NSAIDs (provide common names), clopidogrel (Plavix), warfarin, heparin?
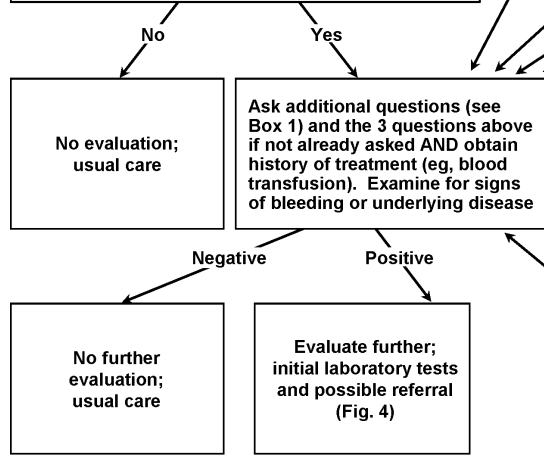
History From Patient

Personal history of VWD

Abnormal laboratory test

Positive family history of a bleeding disorder or bleeding

Patient is concerned about bleeding; patient who has unexplained anemia or history of previous DDAVP use

No / **Yes**

No evaluation; usual care

Ask additional questions (see Box 1) and the 3 questions above if not already asked AND obtain history of treatment (eg, blood transfusion). Examine for signs of bleeding or underlying disease

Negative / **Positive**

No further evaluation; usual care

Evaluate further; initial laboratory tests and possible referral (Fig. 4)

Box 1. Additional Questions to Screen for A Bleeding Disorder

1. Do you have a blood relative who has a bleeding disorder, such as von Willebrand disease or hemophilia?
2. Have you ever had prolonged bleeding from trivial wounds, lasting more than 15 minutes or recurring spontaneously during the 7 days after the wound?
3. Have you ever had heavy, prolonged, or recurrent bleeding after surgical procedures, such as tonsillectomy?
4. Have you ever had bruising, with minimal or no apparent trauma, especially if you could feel a lump under the bruise?
5. Have you ever had a spontaneous nosebleed that required more than 10 minutes to stop or needed medical attention?
6. Have you ever had heavy, prolonged, or recurrent bleeding after dental extractions that required medical attention?
7. Have you ever had blood in your stool, unexplained by a specific anatomic lesion (such as an ulcer in the stomach or polyp in the colon), that required medical attention?
8. Have you ever had anemia requiring treatment or received a blood transfusion?
9. For women, have you ever had heavy menses, characterized by the presence of clots greater than an inch in diameter and/or changing a pad or tampon more than hourly or resulting in anemia or low iron level?

Fig. 3. Initial evaluation of patients to determine whether laboratory testing is indicated. The initial strategy is to determine which patients would benefit most from further diagnostic evaluation for von Willebrand disease (VWD) or other bleeding disorders. Left upper box, Individuals (for example, an asymptomatic person who will undergo a surgical or other invasive procedure) would be asked three questions about their personal and family bleeding history, which, if any responses are positive, would lead to a second set of questions selected for sensitivity and specificity for VWD (lower right, Box 1). Those patients answering positively to one or more of the second set of questions would benefit from laboratory evaluation. Right upper boxes, Patients presenting with specific information or a concern about bleeding would be asked the additional questions (Box 1) and the initial questions (left upper box) if not already asked and would also undergo laboratory evaluation. The three initial questions and the nine additional questions (Box 1) also appear in Table 9 and in Diagnostic Recommendations I. DDAVP, desmopressin; NSAID, non-steroidal anti-inflammatory drug.

common in healthy populations (Table 7, 'Normals' column). Responses to questionnaires used to survey healthy controls indicate that they identify themselves as having specific bleeding manifestations as frequently as persons with VWD, particularly type 1 VWD (Table 7) [134–137]. In addition, a family history of bleeding was reported in 44% of healthy children undergoing tonsillectomy [137] and by 35% [135] or 60% [138] of persons referred because of bleeding. Because bleeding symptoms are so prevalent, it may be impossible to establish a causal relationship between bleeding and low VWF.

Some of the most important clinical issues in VWD apply specifically to women, particularly menorrhagia. Studies of women with VWD report a high prevalence of menorrhagia (Table 7), although the

definition of menorrhagia is not clearly specified in most of these studies and the diagnostic criteria for VWD are not uniform. The sensitivity of menorrhagia as a predictor of VWD may be estimated as 32–100%. However, menorrhagia is a common symptom, occurring with a similar frequency in healthy controls and women with VWD; therefore, it is not a specific marker for VWD (Table 7). In a survey of 102 women who had VWD and were registered at haemophilia treatment centres in the United States, 95% reported a history of menorrhagia, but 61% of controls also reported a history of menorrhagia [139]. Studies have reported a prevalence of VWD of between 5% and 20% among women who have menorrhagia [140–146]. Therefore, the specificity of menorrhagia as a predictor of

Table 7. Common bleeding symptoms of healthy individuals and patients with VWD.

Symptoms	Normals (n = 500 [134]; n = 341 [135]*; n = 88 [148][†]; n = 60 [136][†]), %	All types of VWD (n = 264 [134]; n = 1885 [394]), %	Type 1 VWD (n = 42 [395][‡]; n = 671 [133]), %	Type 2 VWD (n = 497 [133]), %	Type 3 VWD (n = 66 [133]; n = 385 [87]), %
Epistaxis	4.6–22.7	38.1–62.5	53–61	63	66–77
Menorrhagia[§]	23–68.4	47–60	32	32	56–69
Bleeding after dental extraction	4.8–41.9	28.6–51.5	17–31	39	53–70
Ecchymoses	11.8–50	49.2–50.4	50	NR	NR
Bleeding from minor cuts or abrasions	0.2–33.3	36	36	40	50
Gingival bleeding	7.4–47.1	26.1–34.8	29–31	35	56
Postoperative bleeding	1.4–28.2	19.5–28	20–47	23	41
Haemarthrosis	0–14.9	6.3–8.3	2–3	4	37–45
Gastrointestinal bleeding	0.6–27.7	14	5	8	20

NR, not reported; VWD, von Willebrand disease.
*A total of 341 individuals were sent a questionnaire, but the precise number responding was not provided.
[†]Study included women only.
[‡]Study included males only.
[§]Calculated for females older than 13–15 years.

VWD can be estimated as 5–20%. Three findings that predict abnormal menstrual blood loss of more than 80 mL are (i) clots larger than approximately 1 inch in diameter; (ii) low serum ferritin and (iii) the need to change a pad or tampon more than hourly [147].

Further evaluation for inherited bleeding disorders
Because 'bleeding symptoms' other than menorrhagia are reported frequently by persons who have apparently normal haemostasis, it is important to ask questions that can best identify persons who have a true bleeding disorder. Sramek and colleagues [135] used a written questionnaire with patients who had a proven bleeding disorder. When the responses were compared with those of a group of healthy volunteers, the most informative questions were related to (i) prolonged bleeding after surgery, including after dental extractions and (ii) identification of family members who have an established bleeding disorder (Table 8, columns 2–5). A history of muscle or joint bleeding (haematomas or haemarthroses) may also be helpful when associated with the above symptoms.

General questions that relate to isolated bleeding symptoms — such as frequent gingival bleeding, profuse menstrual blood loss, bleeding after delivery and epistaxis in the absence of other bleeding symptoms — were not informative [135]. The study also found that an elaborate interview after referral to a haematologist was not particularly helpful when attempting to distinguish persons who have a true bleeding disorder from persons who have a 'suspected' bleeding disorder, implying that the selection of those with bleeding disorders had already been made by the referring doctor [135].

Drews *et al.* [148] attempted to develop a questionnaire-based screening tool to identify women who might benefit from a diagnostic work-up for VWD. They conducted a telephone survey of 102 women who had a diagnosis of type 1 VWD and were treated at a haemophilia treatment centre; 88 of their friends served as controls. With the exception of postpartum transfusions, all study variables were reported more frequently by women who had VWD than by their friends (Table 8, columns 6 and 7). In addition, positive responses to multiple questions were more likely to be obtained from patients who have an inherited bleeding disorder [148]. An important limitation of this study is that these women were more symptomatic than most women who have a diagnosis of type 1 VWD, indicating a more severe phenotype of the disease; this fact might decrease the sensitivity of the questions in persons who have milder type 1 VWD and fewer symptoms.

More recently, Rodeghiero and colleagues [149] compared responses to a standardized questionnaire obtained from 42 obligatory carriers of VWD (from well-characterized families) to responses from 215 controls. The questionnaire covered 10 common bleeding symptoms (including all symptoms in Table 7 and postpartum haemorrhage), with assigned scores for each ranging from 0 (no symptoms) to 3 (severe symptoms, usually including hospitalization, transfusion support or both). With this instrument, the researchers found that having a cumulative total bleeding score of 3 in men or 5 in

Table 8. Prevalences of characteristics in patients with diagnosed bleeding disorders vs. healthy controls.

Symptom	Univariate analysis*		Multivariate analysis*		Women who have VWD[†]		Type 1 VWD families[‡]	
	OR	95% CI	OR	95% CI	Sensitivity	95% CI	OR	95% CI
Family members have an established bleeding disorder	97.5	38.3–248	50.5	12.5–202.9	…	…	…	…
Profuse bleeding from small wounds	67.2	28.4–159	30.0	8.1–111.1	…	…	16.7	2.0–137.7
Profuse bleeding at site of tonsillectomy/adenoidectomy	27.7	8.0–96.1	11.5	1.2–111.9	…	…	…	…
Easy bruising	12.7	8.0–20.2	9.9	3.0–32.3	9.8	4.8–17.3	8.1	2.1–30.5
Profuse bleeding after surgery	23.0	10.6–50.1	5.8	1.3–26.4	52.9	42.8–62.9	8.9	3.6–21.8
Muscle bleeding (ever)	13.3	6.4–27.7	4.8	0.7–31.4	9.8	4.8–17.3	…	…
Frequent nosebleeds	3.5	2.0–6.2	3.8	0.9–15.7	61.8	51.6–71.2	4.9	2.4–10.0
Profuse bleeding at site of dental extraction	39.4	20.6–75.5	3.2	0.9–11.3	54.9	44.7–64.8	4.6	2.5–8.4
Blood in stool (ever)	2.8	1.7–4.6	2.8	0.7–11.7	13.7	7.7–22.0	1.6	0.6–4.3
Family members with bleeding symptoms	28.6	15.0–54.6	2.5	0.7–9.4	…	…	…	…
Joint bleeding (ever)	8.6	4.8–15.2	2.5	0.6–10.2	20.6	13.2–29.7	…	…
Menorrhagia	5.4	3.0–9.8	2.5	0.6–9.9	…	…	5.1	2.6–10.1
Haemorrhage at time of delivery	5.3	2.3–12.0	2.1	0.3–13.5	50.0	39.9–60.1	0.9	0.3–3.2
Frequent gingival bleeding	2.8	1.9–4.2	0.7	0.3–2.0	76.5	67.0–84.3	1.3	0.3–6.7
Haematuria (ever)	3.2	1.8–5.6	0.5	0.1–2.3	…	…	…	…

CI, confidence interval; OR, odds ratio; VWD, von Willebrand disease.
Ellipses indicate data were not reported.
*Univariate and multivariate analyses from Sramek et al. [135] comparing 222 patients who had a known bleeding disorder (43% mild VWD) and 341 healthy volunteers.
[†]Compiled from responses to a questionnaire sent to 102 women, who had type 1 VWD, in a haemophilia treatment centre, from Drews et al. [148].
[‡]Compiled from interviews comparing affected and unaffected family members of patients who had type 1 VWD, from Tosetto et al. [150] and F. Rodeghiero (personal communication). The index cases (patients who had VWD) were not included in the analysis.

women was very specific (98.6%) but not as sensitive (69.1%) for type 1 VWD. Limitations of this study include its retrospective design and awareness of the respondent's diagnosis by the person administering the questionnaire. This questionnaire is available online [149].

A similar retrospective case–control study [150] used a standardized questionnaire like that of Rodeghiero et al. [149] to compare bleeding symptoms of 144 index cases who had type 1 VWD with those in 273 affected relatives, 295 unaffected relatives and 195 healthy controls. The interviewers were not blinded to subject's status. At least one bleeding symptom was reported by approximately 98% of index cases, 89% of affected relatives, 32% of unaffected relatives and 12% of healthy controls. The major symptoms of affected persons (excluding index cases) included bleeding after tooth extraction, nosebleeds, menorrhagia, bleeding into the skin, postoperative bleeding and bleeding from minor wounds. Using a bleeding score calculated from the

data for comparison, the severity of bleeding diminished with increasing plasma VWF, not only for subjects who had low VWF levels, but throughout the normal range as well. Although the mean bleeding score was significantly different among several groups, the distribution was sufficiently broad that the bleeding score could not predict the affected or unaffected status of individuals.

In a related study, bleeding symptoms were assessed with the same questionnaire in 70 persons who were obligatory carriers of type 3 VWD, 42 persons who were obligate carriers of type 1 VWD (meaning affected family members of index cases who had type 1 VWD) and 215 persons who were healthy controls [151]. Carriers of type 3 VWD were compared with carriers of type 1 VWD to address the question of whether the distinct types of VWF mutations associated with these conditions predisposed to the same or different severity of bleeding. Approximately 40% of carriers of type 3 VWD, 82% of carriers of type 1 VWD and 23% of healthy

controls had at least one bleeding symptom. The major bleeding symptoms in carriers of type 3 VWD were bleeding into skin and postsurgical bleeding. The results suggest that carriers of type 3 VWD are somewhat distinct, because they have bleeding symptoms more frequently than healthy controls but less frequently than persons who have or are carriers of type 1 VWD. Usually, carriers of type 1 VWD have lower VWF levels than carriers of type 3 VWD.

Family history Although a family history that is positive for an established bleeding disorder is useful in identifying persons who are likely to have VWD, such a history is frequently not present. This is most commonly the case for persons with milder forms of VWD and whose family members may have minimal, if any, symptoms. As shown in Table 8, the presence of a documented bleeding disorder in a family member is extremely helpful in deciding which persons to evaluate further, whereas a family history of bleeding symptoms is less helpful.

Summary of medical history evaluation Table 9 summarizes suggested questions that can be used to identify persons who should be considered for further evaluation for VWD with laboratory studies.

Physical examination The physical examination should be directed to confirm evidence for a bleeding disorder, including size, location and distribution of ecchymoses (e.g. truncal), haematomas, petechiae and other evidence of recent bleeding. The examination should also focus on findings that may suggest other causes of increased bleeding, such as evidence of liver disease (e.g. jaundice), splenomegaly, arthropathy, joint and skin laxity (e.g. Ehlers-Danlos syndrome), telangiectasia (e.g. hereditary haemorrhagic telangiectasia), signs of anaemia or anatomic lesions on gynaecological examination.

Acquired von Willebrand Syndrome Persons with AVWS present with bleeding symptoms similar to those described above, except that the past personal history and family history are negative for bleeding symptoms. AVWS may occur spontaneously or in association with other diseases, such as monoclonal gammopathies, other plasma cell dyscrasias, lymphoproliferative diseases, myeloproliferative disorders (e.g. essential thrombocythemia), autoimmune disorders, valvular and congenital heart disease, certain tumours and hypothyroidism [114,152]. The evaluation should be tailored to finding conditions associated with AVWS.

Table 9. Suggested questions for screening persons for a bleeding disorder.

Initial Questions*
1. Have you or a blood relative ever needed medical attention for a bleeding problem, or have you been told you have a bleeding disorder or problem?
   During or after surgery?
   With dental procedures or extractions?
   With trauma?
   During childbirth or for heavy menses?
   Ever had bruises with lumps?
2. Do you have or have you ever had
   Liver or kidney disease?
   A blood or bone marrow disorder?
   A high- or low-platelet count?
3. Are you currently taking or have you recently taken anticoagulation or antiplatelet medications (warfarin, heparin, aspirin, NSAIDs, clopidogrel – provide common names)?
If answers to any of the questions above are positive, obtain relevant specific information including treatment history (e.g. blood transfusions), and ask the following additional questions.

Additional Questions[†‡]
1. Do you have a blood relative who has a bleeding disorder, such as von Willebrand disease or haemophilia?
2. Have you ever had prolonged bleeding from trivial wounds, lasting more than 15 min or recurring spontaneously during the 7 days after the wound?
3. Have you ever had heavy, prolonged, or recurrent bleeding after surgical procedures, such as tonsillectomy?
4. Have you ever had bruising, with minimal or no apparent trauma, especially if you could feel a lump under the bruise?
5. Have you ever had a spontaneous nosebleed that required more than 10 min to stop or needed medical attention?
6. Have you ever had heavy, prolonged, or recurrent bleeding after dental extractions that required medical attention?
7. Have you ever had blood in your stool, unexplained by a specific anatomic lesion (such as an ulcer in the stomach or a polyp in the colon), that required medical attention?
8. Have you ever had anaemia requiring treatment or received a blood transfusion?
9. For women, have you ever had heavy menses, characterized by the presence of clots greater than an inch in diameter or changing a pad or tampon more than hourly or by resulting in anaemia or low iron level?

NSAIDs, non-steroidal anti-inflammatory drugs.
*Initial questions, such as for an asymptomatic person who will undergo a surgical or interventional procedure.
[†]Additional questions such as for persons answering positively to the initial questions or for persons presenting with specific issues, including (i) current bleeding symptoms or a history of increased bleeding; (ii) abnormal laboratory test results; (iii) family history of a bleeding disorder; or (iv) previous diagnosis of a bleeding disorder, including von Willebrand disease. The initial questions should also be asked, if not already done.
[‡]Sources: Laffan *et al.* [8], Drews *et al.* [148] and Dean *et al.* [158].

Fig. 4. Laboratory assessment for von Willebrand disease (VWD) or other bleeding disorders. If the clinical evaluation suggests a bleeding disorder, the initial haemostasis tests should be ordered, followed by or along with the initial VWD assays indicated in the algorithm. Referral to a haemostasis specialist is appropriate for help in interpretation, repeated testing and specialized tests. CBC, complete blood cell count; FVIII, factor VIII; PT, prothrombin time; PTT, partial thromboplastin time; RIPA, ristocetin-induced platelet aggregation; TT, thrombin time; VWF, von Willebrand factor; VWF:Ag, von Willebrand factor antigen; VWF:RCo, von Willebrand factor ristocetin cofactor activity. *Correction of the PTT mixing study immediately and after 2-h incubation removes a FVIII inhibitor from consideration. Investigation of other intrinsic factors and lupus anticoagulant may also be indicated. † Isolated decreased platelets may occur in VWD type 2B.
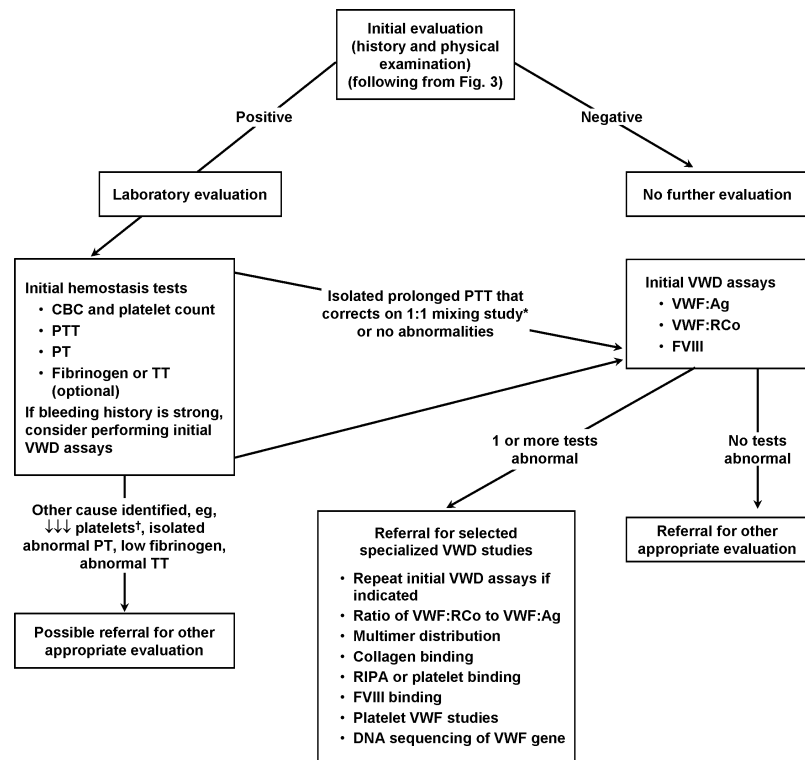
Laboratory diagnosis and monitoring

An algorithm for using clinical laboratory studies to make the diagnosis of VWD is summarized in Fig. 4.

Ideally, a simple, single laboratory test could screen for the presence of VWD. Such a screening test would need to be sensitive to the presence of most types of VWD and would have a low false-positive rate. However, no such test is available. In the past, the activated partial thromboplastin time (PTT) and BT were recommended as diagnostic tests. These tests were probably satisfactory for detecting severe type 3 VWD, but as variant VWD and milder forms of VWD were characterized, it became apparent that many of the persons who have these conditions had normal PTT and BT results.

Initial haemostasis laboratory evaluation An initial haemostasis laboratory evaluation usually includes (i) a platelet count and complete blood cell count; (ii) PTT; (iii) prothrombin time; and (iv) optionally either a fibrinogen level or a thrombin time (Fig. 4). This testing neither rules in nor rules out VWD, but it can suggest whether coagulation factor deficiency or thrombocytopenia might be the potential cause of clinical bleeding. If the mucocutaneous bleeding history is strong, initial VWD assays (VWF:Ag, VWF:RCo and FVIII) should be considered at the first visit.

Some centres add a BT or a platelet function analyzer (PFA-100) assay to their initial laboratory tests. The BT test is a non-specific test and subject to operational variation. It has been argued that the BT test is a population-based test that was never intended to test individuals [153]. Variables that may affect results include a crying or wiggling child, differences in the application of the blood pressure cuff, and the location, direction and depth of the cut made by the device. This test also has a potential for causing keloid formation and scarring, particularly in non-White individuals.

The PFA-100 test result has been demonstrated to be abnormal in the majority of persons with VWD, other than those with type 2N, but its use for population screening for VWD has not been established [154–157]. Persons with severe type 1 VWD or type 3 VWD usually have abnormal PFA-100 values, whereas persons with mild or moderate type 1 VWD and some with type 2 VWD may not have abnormal results [158–160]. When persons are studied by using both the BT and PFA-100, the results are not always concordant [157,159,161].

When using the PTT in the diagnosis of VWD, results of this test are abnormal only if the FVIII is sufficiently reduced. Because the FVIII gene is normal

in VWD, the FVIII deficiency is secondary to the deficiency of VWF, its carrier protein. In normal individuals, the levels of FVIII and VWF:RCo are approximately equal, with both averaging 100 IU dL^{-1}. In type 3 VWD, the plasma FVIII level is usually <10 IU dL^{-1} and represents the steady state of FVIII in the absence of its carrier protein. In persons with type 1 VWD, the FVIII level is often slightly higher than the VWF level and may fall within the normal range. In persons with type 2 VWD (except for type 2N VWD in which it is decreased), the FVIII is often two to three times higher than the VWF activity (VWF:RCo) [162,163]. Therefore, the PTT is often within the normal range. If VWF clearance is the cause of low VWF, the FVIII reduction parallels that of VWF, probably because both proteins are cleared together as a complex.

Initial tests for VWD The initial tests commonly used to detect VWD or low VWF are determinations of plasma levels of (i) VWF:Ag; (ii) VWF:RCo; and (iii) FVIII (Fig. 4). These three tests, readily available in most larger hospitals, measure the amount of VWF protein present in plasma (VWF:Ag), the function of the VWF protein that is present as VWF:RCo, and the ability of the VWF to serve as the carrier protein to maintain normal FVIII survival. If any of the above tests is abnormally low, the next steps should be discussed with a coagulation specialist, who may recommend referral to a specialized centre and repeating the initial VWD laboratory tests in addition to performing other tests.

VWF:Ag assay. VWF:Ag is an immunoassay that measures the concentration of VWF protein in plasma. Commonly used methods are based on enzyme-linked immunosorbent assay (ELISA) or automated latex immunoassay (LIA). As discussed below, the standard reference plasma is critical and should be keyed to the World Health Organization (WHO) standard. The person's test results should be reported in international units (IU), either as international units per decilitre (IU dL^{-1}) or as international units per millilitre (IU mL^{-1}). Most laboratories choose IU dL^{-1}, because it is similar to the conventional manner of reporting clotting factor assays as a percentage of normal.

VWF:RCo assay. VWF:RCo is a functional assay of VWF that measures its ability to interact with normal platelets. The antibiotic ristocetin causes VWF to bind to platelets, resulting in platelet clumps and their removal from the circulation. Ristocetin was removed from clinical trials because it caused thrombocytopenia. This interaction was developed into a laboratory test that is still the most widely accepted functional test for VWF. (*In vivo*, however, it is the high shear in the microcirculation, and not a ristocetin-like molecule, that causes the structural changes in VWF that lead to VWF binding to platelets.)

Several methods are used to assess the platelet agglutination and aggregation that result from the binding of VWF to platelet GPIb induced by ristocetin (VWF:RCo). The methods include (1) time to visible platelet clumping using ristocetin, washed normal platelets (fresh or formalinized) and dilutions of patient plasma; (2) slope of aggregation during platelet aggregometry using ristocetin, washed normal platelets and dilutions of the person's plasma; (3) automated turbidometric tests that detect platelet clumping, using the same reagents noted above; (4) ELISAs that assess direct binding of the person's plasma VWF to platelet GPIb (the GPIb may be derived from plasma glycocalicin) in the presence of ristocetin [164–166] and (5) the binding of a monoclonal antibody to a conformation epitope of the VWF A1 loop [167]. Method (5) can be performed in an ELISA or LIA format. It is not based on ristocetin binding. The first three assays above may use platelet membrane fragments containing GPIb rather than whole platelets. The sensitivity varies for each laboratory and each assay; in general, however, methods 1 and 2, which measure platelet clumping by using several dilutions of the person's plasma, are quantitative to approximately 6–12 IU dL^{-1} levels. Method 3 is quantitative to about 10–20 IU dL^{-1}. Method 4 can measure VWF:RCo to <1 IU dL^{-1}, and a variation of it can detect the increased VWF binding to GPIb seen in type 2B VWD [168]. Some automated methods are less sensitive and require modification of the assay to detect levels <10 IU dL^{-1}. Each laboratory should define the linearity and limits of its assay. Several monoclonal ELISAs (method 5) that use antibodies directed to the VWF epitope containing the GPIb-binding site have been debated because the increased function of the largest VWF multimers is not directly assessed [169].

The VWF:RCo assay has high intralaboratory and interlaboratory variation, and it does not actually measure physiological function. The coefficient of variation (CV) has been measured in laboratory surveys at 30% or greater, and the CV is still higher when the VWF:RCo is lower than 12–15 IU dL^{-1} [170–174]. This becomes important not only for the initial diagnosis of VWD, but also for determining whether the patient has type 1 vs. type 2 VWD (see additional discussion in the section below, Ratio of VWF:RCo to VWF:Ag). Despite these limitations, it

is still the most widely accepted laboratory measure of VWF function. Results for VWF:RCo should be expressed in IU dL^{-1} on the basis of the WHO plasma standard.

FVIII assay. FVIII coagulant assay is a measure of the cofactor function of the clotting factor, FVIII, in plasma. In the context of VWD, FVIII activity measures the ability of VWF to bind and maintain the level of FVIII in the circulation. In the United States, the assay is usually performed as a 1-stage clotting assay based on the PTT, although some laboratories use a chromogenic assay. The clotting assay, commonly done using an automated or semiautomated instrument, measures the ability of plasma FVIII to shorten the clotting time of FVIII-deficient plasma. Because this test is important in the diagnosis of haemophilia, the efforts to standardize this assay have been greater than those applied to other haemostasis assays. FVIII activity is labile, with the potential for spuriously low assay results if blood

specimen collection, transport or processing is suboptimal. Like the tests discussed above, it should be expressed in IU dL^{-1} on the basis of the WHO plasma standard.

Laboratory results in different VWD subtypes Expected patterns of laboratory results in different subtypes of VWD, depicted in Fig. 5, include results of the three initial VWD tests (VWF:Ag, VWF:RCo and FVIII) and results of other assays for defining and classifying VWD subtypes. The three initial tests (or at least the VWF:RCo and FVIII assays) are also used for monitoring therapy.

Other assays to measure VWF, define and diagnose VWD and classify subtypes

VWF multimer analysis. The VWF multimer test, an assay that is available in some larger centres and in commercial laboratories, is usually performed after

	Normal	Type 1	Type 2A	Type 2B	Type 2M	Type 2N	Type 3	PLT-VWD*
VWF:Ag	N	L, ↓ or ↓↓	↓ or L	↓ or L	↓ or L	N or L	Absent	↓ or L
VWF:RCo	N	L, ↓ or ↓↓	↓↓ or ↓↓↓	↓↓	↓↓	N or L	Absent	↓↓
FVIII	N	N or ↓	N or ↓	N or ↓	N or ↓	↓↓	1-9 IU/dL	N or L
RIPA	N	Often N	↓	Often N	↓	N	Absent	Often N
LD-RIPA	Absent	Absent	Absent	↑↑↑	Absent	Absent	Absent	↑↑↑
PFA-100 CT	N	N or ↑	↑	↑	↑	N	↑↑↑	↑
BT	N	N or ↑	↑	↑	↑	N	↑↑↑	↑
Platelet count	N	N	N	↓ or N	N	N	N	↓
VWF multimer pattern	N	N	Abnormal	Abnormal	N	N	Absent	Abnormal

Fig. 5. Expected laboratory values in von Willebrand disease (VWD). The symbols and values represent prototypical cases. In practice, laboratory studies in certain patients may deviate slightly from these expectations. L, 30–50 IU dL^{-1}; ↓, ↓↓, ↓↓↓, relative decrease; ↑, ↑↑, ↑↑↑, relative increase; BT, bleeding time; FVIII, factor VIII activity; GPIb, platelet glycoprotein Ib complex; LD-RIPA, low-dose ristocetin-induced platelet aggregation (concentration of ristocetin ≤0.6 mg mL^{-1}); N, normal; PFA-100-CT, platelet function analyzer closure time; PLT-VWD, platelet-type VWD; RIPA, ristocetin-induced platelet aggregation; VWF, von Willebrand factor; VWF:Ag, von Willebrand factor antigen; VWF:RCo, von Willebrand factor ristocetin cofactor activity. (Courtesy of R. R. Montgomery, the BloodCenter of Wisconsin and Medical College of Wisconsin, Milwaukee, Wisconsin; adapted from and used with permission.) *Persons with PLT-VWD have a defect in their platelet GPIb. Laboratory test results resemble type 2B VWD, and both have a defect in their LD-RIPA. In the VWF platelet-binding assay, persons with type 2B VWD have abnormally increased platelet binding. Normal persons and those with PLT-VWD have no binding of their VWF to normal platelets at low ristocetin concentrations.

the initial VWD testing indicates an abnormality, preferably using a previously unthawed portion of the same sample or in association with a repeated VWD test panel (VWF:Ag, VWF:RCo and FVIII) using a fresh plasma sample. VWF multimer analysis is a qualitative assay that depicts the variable concentrations of the different-sized VWF multimers by using sodium dodecyl sulphate–protein electrophoresis followed by detection of the VWF multimers in the gel, using a radiolabelled polyclonal antibody or a combination of monoclonal antibodies. Alternatively, the protein is transferred to a membrane (Western blot), and the multimers are identified by immunofluorescence or other staining techniques [101,175,176].

Multimer assays are designated as 'low resolution' (which differentiate the largest multimers from the intermediate and small multimers) or 'high resolution' (which differentiate each multimer band of the smaller multimers into 3–8 satellite bands). For diagnostic purposes, the low-resolution gel systems are used primarily; these systems help to differentiate the type 2 VWD variants from types 1 or 3 VWD. Figure 6 illustrates the differences between these two techniques with regard to the resolution of high- and low-molecular-weight multimers. It should be noted that multimer appearance alone does not define the variant subtype and that only types 2A, 2B and platelet-type VWD (PLT-VWD) have abnormal multimer distributions with relative deficiency of the largest multimers. An exception is Vicenza variant VWD with ultralarge VWF multimers and low VWF. For more information about VWF multimer findings in type 2 VWD variants, see
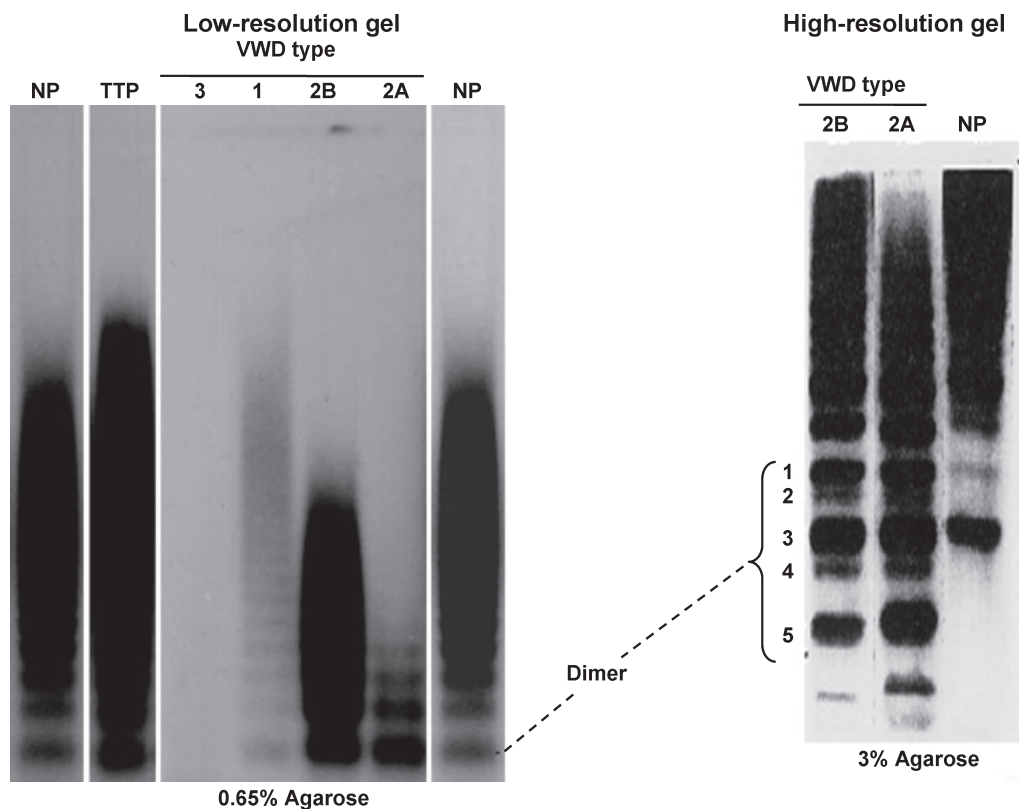

Fig. 6. Analysis of von Willebrand factor (VWF) multimers. The distribution of VWF multimers can be analysed using sodium dodecyl sulphate (SDS)–agarose electrophoresis followed by immunostaining. Low-resolution gels (0.65% agarose, left side) can demonstrate the change in multimer distribution of the larger multimers (top of the gel), while high-resolution gels (2–3% agarose, right side) can separate each multimer into several bands that may be distinctive. For example, the lowest band in the 0.65% gel can be resolved into five bands in the 3% agarose gel, but the 3% gel fails to demonstrate the loss of high-molecular-weight multimers seen at the top in the 0.65% gel. The dotted line indicates the resolution of the smallest band into several bands in the 3% agarose gel. In each gel, normal plasma (NP) is run as a control. Type 1 von Willebrand disease (VWD) plasma has all sizes of multimers, but they are reduced in concentration. Type 2A VWD plasma is missing the largest and intermediate multimers, while type 2B VWD plasma is usually missing just the largest VWF multimers. No multimers are identified in type 3 VWD plasma. Patients with thrombotic thrombocytopenic purpura (TTP) may have larger than normal multimers when studied with low-resolution gels. (Courtesy of R. R. Montgomery, the BloodCenter of Wisconsin and Medical College of Wisconsin, Milwaukee, Wisconsin; used with permission).

the section above (Type 2 VWD) and associated references.

Low-dose RIPA. RIPA and the VWF platelet-binding (VWF:PB) assay are two tests that are performed to aid in diagnosing type 2B VWD. RIPA may be done as part of routine platelet aggregation testing. Low-dose RIPA is carried out in platelet-rich plasma, using a low concentration of ristocetin (usually <0.6 mg mL^{-1}, although ristocetin lots vary, resulting in the use of slightly different ristocetin concentrations). This low concentration of ristocetin does not cause VWF binding and aggregation of platelets in samples from normal persons, but it does cause VWF binding and aggregation of platelets in samples from patients with either type 2B VWD or mutations in the platelet VWF receptor. The latter defects have been termed pseudo-VWD or PLT-VWD, and they can be differentiated from type 2B VWD by VWF:PB assay. At higher concentrations of ristocetin (1.1–1.3 mg mL^{-1}), RIPA is reduced in persons with type 3 VWD. However, the test is not sufficiently sensitive to reliably diagnose other types of VWD.

VWF:PB assay. The VWF:PB assay measures the binding of VWF to normal paraformaldehyde-fixed platelets using low concentrations of ristocetin (usually 0.3–0.6 mg mL^{-1}) [177]. The amount of VWF bound to the fixed platelets is determined by using a labelled antibody. Clinically normal individuals or those with types 1, 2A, 2M, 2N and 3 VWD exhibit minimal or no binding to platelets at the concentration of ristocetin used, but patients with type 2B VWD exhibit extensive binding that causes their variant phenotype (a loss of high-molecular-weight multimers, decreased ristocetin cofactor activity, and thrombocytopenia). Both type 2B VWD and PLT-VWD have agglutination of platelet-rich plasma to low-dose ristocetin, but the VWF:PB assay can differentiate type 2B VWD from PLT-VWD. Only VWF from persons with type 2B VWD has increased VWF:PB, while VWF from persons with PLT-VWD has normal VWF:PB with low doses of ristocetin.

VWF collagen-binding assay. The VWF collagen-binding (VWF:CB) assay measures binding of VWF to collagen. The primary site of fibrillar collagen binding is in the A3 domain of VWF. Like the ristocetin cofactor assay, the VWF:CB assay is dependent on VWF multimeric size, with the largest multimers binding more avidly than the smaller forms. The VWF:CB assay performance and sensitivity to VWD detection or discrimination among VWD subtypes is highly dependent on the source of collagen, as well as on whether type 1 collagen or a mixture of type 1/3 collagen is used [178,179]. Only

a few patients have been identified who have specific collagen-binding defects that are independent of multimer size, and the defects have been associated with a mutation of VWF in the A3 domain [75]. The prevalence of such defects is unknown. The place of VWF:CB in the evaluation of VWD has not been established. In principle, however, patients who have defects in collagen binding may have a normal VWF:RCo and thus escape clinical diagnosis unless a VWF:CB assay is performed. Limited studies suggest that supplementary VWF:CB testing, complementing assays of VWF:RCo and VWF:Ag, can improve the differentiation of type 1 VWD from types 2A, 2B or 2M VWD [170,180,181].

VWF FVIII-binding assay. The VWF FVIII-binding (VWF:FVIIIB) assay measures the ability of a person's VWF to bind added exogenous FVIII and is used to diagnose type 2N VWD [77,79,80,182,183]. The assay is performed by capturing the person's VWF on an ELISA plate, removing the bound endogenous FVIII, and then adding back a defined concentration of exogenous recombinant FVIII. The amount of FVIII bound is determined by chromogenic or immunological FVIII assay. The level of this bound FVIII is then related to the amount of the person's VWF initially bound in the same well. In clinical experience, type 2N VWD is usually recessive; the person is either homozygous or compound heterozygous (one allele is type 2N and the other is a type 1 or null allele). In either case, the VWF in the circulation does not bind FVIII normally, and the concentration of FVIII is thus decreased.

Ratio of VWF:RCo to VWF:Ag. The VWF:RCo/VWF:Ag ratio can aid in the diagnosis of types 2A, 2B and 2M VWD and help differentiate them from type 1 VWD. For example, a VWF:RCo/VWF:Ag ratio of <0.6 [184] or 0.7 has been used as a criterion for dysfunctional VWF [8,185]. A similar approach has been proposed for the use of the VWF:CB/VWF:Ag ratio [8,185]. In type 2A VWD, the ratio is usually low, and in type 2B VWD, the VWF:RCo/VWF:Ag ratio is usually low but may be normal. In type 2M VWD, the VWF:Ag concentration may be reduced or normal, but the VWF:RCo/VWF:Ag ratio is <0.7. One study [72] determined the VWF:RCo/VWF:Ag ratio in nearly 600 individuals with VWF levels lower than 55 IU dL^{-1} who had normal VWF multimers. The study used this ratio to identify families who had type 2 VWD, but most centres do not have the ability to establish normal ranges for patients who have low VWF. Additionally, the VWF:RCo assay has a CV as high as 30% or more, depending on the methods used, whereas the CV for the VWF:Ag assay is somewhat lower. The high

intrinsic variability of the VWF:RCo assay, especially at low levels of VWF, can make the VWF:RCo/VWF:Ag ratio an unreliable criterion for the diagnosis of type 2 VWD [170,172–174].

It is important that the same plasma standard used in both the VWF:RCo and VWF:Ag assays and that a reference range for the VWF:RCo/VWF:Ag ratio and its sensitivity to types 2A and 2M VWD be determined in each laboratory. Because no large multicentre studies have evaluated the precise ratio that should be considered abnormal, a ratio in the range of less than 0.5–0.7 should raise the suspicion of types 2A, 2B or 2M VWD. Further confirmation should be sought by additional testing (e.g. repeated VWD test panel and VWF multimer study or sequencing of the A1 region of the VWF gene) [186]. Subsequent sections provide more information about the VWF:RCo/VWF:Ag ratio (Diagnostic Recommendations II.C.1.a and III.B and Evidence Table 3 [10]).

ABO blood type. ABO blood types have a notable effect on plasma VWF (and FVIII) concentrations [45,187]. Individuals who have blood type O have concentrations approximately 25% lower than persons who have other ABO blood types. The diagnosis of type 1 VWD occurs more frequently in individuals who have blood group type O [45]. Table 10 illustrates the effect of blood type on VWF:Ag level.

Although stratification of reference ranges for VWF:Ag and VWF:RCo with respect to blood group O and non-group O has been recommended [188,189], limited evolving information supports the concept that, despite the ABO blood grouping and associated VWF reference ranges, the major determinant of bleeding symptoms or risk is low VWF [184,190,191]. Therefore, referencing VWF testing results to the population reference range, rather than to ABO-stratified reference ranges, may be more useful clinically.

Table 10. Influence of ABO blood groups on VWF:Ag.

ABO type	Number	VWF:Ag mean (U dL^{-1})	Range, U dL^{-1} (IU dL^{-1})
O	456	74.8	35.6–157.0 (41–179)
A	340	105.9	48.0–233.9 (55–267)
B	196	116.9	56.8–241.0 (65–275)
AB	109	123.3	63.8–238.2 (73–271)

VWF:Ag, von Willebrand factor antigen.
Modified from Gill *et al.* [45]. Used with permission. In this publication, VWF:Ag was expressed as U dL^{-1} (units per deciliter), but the range in IU dL^{-1} (WHO) is higher for all blood groups, as noted in the values in parentheses (R. R. Montgomery, J. L. Endres and K. D. Friedman, personal communication).

Platelet VWF assays. Platelet VWF studies are performed by some laboratories, including VWF:RCo, VWF:Ag and VWF multimers, using VWF extracted from washed platelets. The methods and interpretations of these studies, however, are not well standardized.

DNA sequencing analysis. DNA sequencing of patient DNA has been used to make a molecular diagnosis of variants of type 2 VWD [192–194], but DNA sequencing is not widely available. Most of the mutations found in types 2B, 2M and 2N VWD cluster in the cDNA that directs the synthesis of specific regions of VWF (Fig. 2) [195]. In the common forms of type 2A VWD, in which the VWF is spontaneously cleaved by ADAMTS13, mutations cluster in the A2 domain (which contains the cleavage site). In the less common type 2A variants of VWD, in which multimer formation is inhibited, the mutations may be scattered throughout the gene. In most persons with type 1 VWD, the genetic mutations have not been established, although several studies are under way to characterize these mutations.

Assays for detecting VWF antibody. Assays for detecting anti-VWF antibodies are not as well established as the assays for detecting antibodies to FVIII in patients with haemophilia A. Some patients with AVWS do appear to have anti-VWF antibodies that decrease the half-life of infused VWF. Although a few antibodies do inhibit VWF function and can be demonstrated in 1:1 mixing studies with normal plasma using the VWF:RCo assay, most anti-VWF antibodies are not inhibitors of VWF function. The presence of these antibodies, however, promotes rapid clearance of VWF. The plasma level of the VWFpp is normally proportionate to the level of VWF:Ag, and the VWFpp level can be measured to aid in the detection of the rapid clearance of VWF. Accelerated plasma clearance of VWF:Ag — as occurs in some patients with AVWS, in those who have certain type 1 VWD variants, or in those who have type 3 VWD and have alloantibodies to VWF — is associated with an increase in the ratio of VWFpp to VWF:Ag [196,197]. Persons with type 3 VWD, with large deletions of the VWF gene, are prone to develop alloantibodies to transfused VWF [198]. Patients who have AVWS, VWF antibodies or mutations that affect VWF clearance can be studied using VWF-survival testing after administration of desmopressin or VWF concentrate.

Making the diagnosis of VWD

Scoring systems and criteria for assessing the bleeding history and the probability of having VWD,

especially type 1 VWD, are evolving and have not yet been subjected to prospective studies outside defined populations [149,189]. Establishing the diagnosis of VWD in persons with type 2 VWD variants and type 3 VWD is usually straightforward, based on the initial VWD tests (VWF:Ag, VWF:RCo and FVIII). Treatment depends on the specific subtype (e.g. type 2A, 2B, 2M or 2N), which is determined by additional tests, including VWF multimer analysis. In contrast, the diagnosis of type 1 VWD is often more difficult [22,46,95,199,200], partly because not all persons who have decreased levels of VWF have a molecular defect in the VWF gene. Whether individuals who do not have an abnormality in the VWF gene should be diagnosed as having VWD or should be given another designation is currently under consideration (see Type 1 VWD vs. Low VWF). The reasons for reduced VWF levels in many of these persons who have a normal VWF gene sequence are not understood. A 'low' VWF level is believed to confer some bleeding risk, despite the presence of a normal VWF gene, and those persons who have clinical bleeding and low VWF concentrations may benefit from treatment to raise the VWF level. Most clinicians would agree that persons having VWF levels <30 IU dL^{-1} probably have VWD. It is likely that most of these persons have a mutation in the VWF gene. Currently, several large European Union, Canadian and US studies are trying to define that frequency. Persons whose plasma VWF levels are below the lower limit of the laboratory reference range, but higher than 30 IU dL^{-1}, may have VWD but are sometimes referred to as having 'possible type 1 VWD' or 'low VWF'. There is no generally accepted designation for these persons. Although type 3 VWD is usually the result of inheriting two null alleles, the heterozygous carriers in these families do not universally have a history of serious bleeding; therefore, type 3 VWD has been called a recessive disorder [22,46,103].

Special considerations for laboratory diagnosis of VWD

Repeated laboratory testing. Repeated testing for VWD is sometimes needed to identify low levels of VWF. Stress — including surgery, exercise, anxiety, crying in a frightened child, as well as systemic inflammation, pregnancy or administration of oestrogen or oral contraceptives — can increase plasma levels of VWF and mask lower baseline values. VWF levels vary with the menstrual cycle, and lowest values are detected on days 1–4 of the menstrual cycle. However, the importance of timing of the testing with respect to the menstrual cycle is not clear. Family studies may be helpful to diagnose hereditary decreases in VWF levels.

Blood sample collection and processing. Problems may occur in preparing samples for testing. As noted above, anxiety may falsely elevate the VWF and FVIII levels, and the setting for phlebotomy should be as calm as possible. It is important that the sample be obtained by atraumatic collection of blood, drawn into the appropriate amount of citrate anticoagulant. The College of American Pathologists, as well as the Clinical Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards, commonly known as NCCLS), recommends collecting blood into 3.2% citrate, although some laboratories still use 3.8% citrate. Fasting or non-lipaemic samples should be used for testing, and icteric or haemolysed samples may also compromise the quality of testing results [188,201]. If a person has polycythemia or profound anaemia, the amount of anticoagulant should be adjusted on the basis of nomograms designed for this purpose. Blood should be centrifuged promptly to obtain plasma, and the plasma should remain at room temperature if assays are to be completed within 2 h. Whole blood should not be transported on wet ice (or frozen) [202,203]. If plasma samples are frozen, they should be thawed at 37°C to avoid formation of a cryoprecipitate. Plasma assays should be performed on platelet-poor or platelet-free plasma [188]. Although a small number of platelets may not greatly affect studies done on fresh plasma, freezing these samples may result in the release of proteases or platelet membrane particles that affect plasma assays for VWF. Thus, plasmas should be centrifuged carefully. Some laboratories perform double centrifugation to ensure platelet removal. The integrity of samples may suffer during transport to an outside laboratory, and steps should be taken that can best ensure prompt delivery of frozen samples. Table 11 provides additional information about collection and processing of blood and plasma samples for laboratory testing.

VWF reference standards. The VWF reference standard is critical to the laboratory diagnosis of VWD. When possible, all laboratory assays of VWF should use the same standard to avoid artifactual discrepancies. Results of VWF assays can be reported in IUs only if they have been referenced to the WHO standard for that analyte. If a reference plasma pool is used, it is usually reported as a percentage of normal, as it cannot be called an IU. To assist the comparison, IUs are usually expressed as IU dL^{-1} so that the reported values have the same range as values reporting a percentage of normal plasma.

Table 11. Collection and handling of plasma samples for laboratory testing.

Phlebotomy conditions – An atraumatic blood draw limits the exposure of tissue factor from the site and the activation of clotting factors, minimizing falsely high or low values

Patient stress level – Undue stress, such as struggling or crying in children or anxiety in adults, may falsely elevate VWF and FVIII levels. Very recent exercise can also elevate VWF levels.

Additional conditions in the person – The presence of an acute or chronic inflammatory illness may elevate VWF and FVIII levels, as may pregnancy or administration of oestrogen or oral contraceptives

Sample processing – To prevent cryoprecipitation of VWF and other proteins, blood samples for VWF assays should be transported to the laboratory at room temperature. Plasma should be separated from blood cells promptly at room temperature, and the plasma should be centrifuged thoroughly to remove platelets. If plasma samples will be assayed within 2 h, they should be kept at room temperature. Frozen plasma samples should be carefully thawed at 37°C and kept at room temperature for <2 h before assay.

Sample storage – Plasma samples that will be stored or transported to a reference laboratory must be frozen promptly at or below −40°C and remain frozen until assayed. A control sample that is drawn, processed, stored and transported under the same conditions as the tested person's sample may be helpful in indicating problems in the handling of important test samples.

FVIII, factor VIII; VWF, von Willebrand factor.

Laboratory testing variables. Laboratory variables also occur. The variability (CV) of the VWF:RCo assay is high (≥20–30%), and the CV of the VWF:Ag assay is also relatively high (≥10–20%), as is the CV for the FVIII assay [170,172–174,178,204–206]. The quality of laboratory testing also varies considerably among laboratories (high interlaboratory CV). Coupled with variability of VWF and FVIII contributed by conditions of the patient and the blood sample, the high variability of these three diagnostic tests can contribute to difficulty in diagnosing VWD or classifying the VWD subtype (e.g. type 1 vs. type 2 variant, using the VWF:RCo/VWF:Ag ratio). Some of the more specialized tests, such as VWF multimer analysis likely also have high variability of test performance and interpretation [175,176], and they are often not available at local testing laboratories.

Summary of the laboratory diagnosis of VWD The diagnosis of VWD can be complex, and no single diagnostic approach is suitable for all patients. Improvements in laboratory testing and quality, along with further research into the frequency of mutations of the VWF gene, alterations of other proteins that result in reduced VWF levels, and the correlation of clinical symptoms with laboratory test levels are necessary to place the diagnosis of VWD on a more secure foundation.

Diagnostic recommendations

The recommendations are graded according to criteria described in Table 1. Diagnostic Recommendations I–III summarize the Panel's patient diagnostic recommendations. Evidence Tables 1–5 are provided in the full online document [10] for recommendations given a grade of B and having two or more references.

The recommendations that follow (Diagnostic Recommendations I, II and III) include specific clinical history, physical findings, laboratory assays, and diagnostic criteria that this Panel suggests will provide the most definitive diagnosis of VWD.

In addition, the Panel suggests the following with regard to laboratory testing (Diagnostic Recommendations II). (i) Tests such as the BT, PFA-100 or other automated functional platelet assays have been used, but there are conflicting data with regard to sensitivity and specificity for VWD [157,159,161]. Therefore, the Panel believes current evidence does not support their routine use as screening tests for VWD. (ii) The Panel believes that platelet-based assays should be used for the ristocetin cofactor method (VWF:RCo assay). (iii) The Panel emphasizes the importance of the timing of the phlebotomy for assays, with the patient at his or her optimal baseline as far as possible. (For example, VWF levels may be elevated above baseline during the second and third trimesters of pregnancy or during oestrogen therapy, during acute inflammation such as the perioperative period, during infections and during acute stress.) The careful handling and processing of the sample are also critical, particularly if the sample will be sent for testing at a remote location.

Laboratory testing should be guided by the history and physical findings (Diagnostic Recommendations I) and the initial laboratory evaluation (Diagnostic Recommendations II). For example, findings of liver disease may lead to a different or additional laboratory evaluation rather than an evaluation for VWD. Diagnostic Recommendations III detail recommendations for synthesis of clinical findings and laboratory test results to make the diagnosis of VWD.

Diagnostic Recommendations I

*I. Evaluation of Bleeding Symptoms and Bleeding Risk by History and Physical Examination**

A. Ask the following broad questions:
   1. Have you or a blood relative ever needed medical attention for a bleeding problem, or have

you been told you had a bleeding problem?
Grade B, level IIb [135]

If the answer is 'Yes' to either of the broad questions above, ask the additional probes:

    a. Have you needed medical attention for bleeding? After surgery? After dental work? With trauma?

    b. Have you ever had bruises so large they had lumps?

Grade B, level IIb [135]

2. Do you have or have you ever had:
    a. Liver or kidney disease?
    b. A blood or bone marrow disorder? A high or low platelet count?

If the answer is 'Yes' to any of these questions, obtain relevant details.
Grade C, level IV

3. Are you currently taking, or have you recently taken anticoagulation or antiplatelet medications (warfarin, heparin, aspirin, nonsteroidal anti-inflammatory drugs, clopidogrel)?
If the answer is 'Yes', obtain relevant details.
Grade C, level IV

B. If answers to questions I.A.1 are positive, ask if the patient or any blood relatives have had the following:

1. A bleeding disorder, such as von Willebrand disease or haemophilia?
2. Prolonged, heavy, or recurrent bleeding from
    a. Trivial wounds, lasting more than 15 min or recurring spontaneously during the 7 days after the wound?
    b. Surgical procedures, such as tonsillectomy?
3. Bruising with minimal or no apparent trauma, especially if you could feel a lump?
4. Spontaneous nosebleeds that required more than 10 min to stop or needed medical attention?
5. Dental extractions leading to heavy, prolonged, or recurrent bleeding?
6. Blood in your stool, unexplained by a specific anatomic lesion (such as an ulcer in the stomach or a polyp in the colon), that required medical attention?
7. Anaemia requiring treatment or received a blood transfusion?
8. For women, heavy menses, characterized by the presence of clots larger than 1 inch and/or changing a pad or tampon more than hourly or resulting in anaemia or low iron level?

If answers to questions I.B.1–8 are positive, obtain relevant specific information, including history of treatment (e.g. blood transfusion).
Grade B, level IIb [135,148]

See Evidence Table 1 [10] for additional detail and information.

C. Perform a physical examination to include evaluation for

1. Evidence for bleeding disorder, including size, location, and distribution of ecchymoses (e.g. truncal), haematomas, petechiae, and other evidence of recent bleeding and/or anaemia.
Grade C, level IV

2. Evidence that suggests other causes or risks of increased bleeding, such as jaundice or spider angiomas (liver disease), splenomegaly, arthropathy, joint and skin laxity (e.g. Ehlers-Danlos syndrome), telangiectasia (e.g. hereditary haemorraghic telangiectasia), or evidence of anatomic lesion on gynaecologic examination.
Grade C, level IV

*Summarized in Fig. 3 and Table 9.

Diagnostic Recommendations II

II. Evaluation by Laboratory Testing*

A. Initial laboratory evaluation for the aetiology of a bleeding disorder should include the following:

1. A complete blood cell count (including platelet count), prothrombin time, activated partial thromboplastin time (PTT), and optionally either thrombin time or fibrinogen level.

2. If laboratory abnormalities besides the PTT are present (the platelet count may also be decreased in type 2B von Willebrand disease [VWD]), in conjunction with the history and physical examination findings, consider bleeding disorders other than VWD or additional underlying diseases.

3. If the mucocutaneous bleeding history is strong, consider performing initial VWD assays at the first visit (see II.B, below).

4. If there are no abnormalities on initial blood testing or if there is an isolated prolonged PTT that corrects in the 1:1 mixing study, the following 3 tests for VWD should be performed (II.B, below), unless another cause for bleeding has been identified and VWD is not likely (see Fig. 4). For further laboratory evaluation, physicians may consider referral to a haemostasis center because of the special sample handling and testing requirements (see Table 11).
Grade C, level IV

B. Initial tests for diagnosing or excluding VWD include the following three tests:

1. Von Willebrand factor ristocetin cofactor activity (VWF:RCo).
2. Von Willebrand factor antigen (VWF:Ag).
3. Factor VIII activity (FVIII).

Grade B, level III [3,45,170,171]

See Evidence Table 2 [10] for additional detail and information.

C. If any of the above test results is abnormally low, a discussion with or a referral to a haemostasis expert is appropriate. In addition to repeating the initial three tests (in most cases), the specialist may recommend appropriate studies from the following:

1. The first set of additional tests may include

a. Evaluation of the ratio of VWF activity (VWF:RCo and/or von Willebrand factor collagen-binding activity [VWF:CB]) to VWF:Ag (only in laboratories that have defined reference ranges for the ratio[s]).

Grade B, level III [72,73,93,158,170,185]

See Evidence Table 3 [10] for additional information and detail.

b. VWF multimer study.

Grade B, level III [176]

c. Ristocetin-induced platelet aggregation (RIPA).

Grade B, level III [48]

d. VWF:CB.

Grade B, level IIb [170,180,181]

See Evidence Table 4 [10] for additional information and detail.

2. Studies in selected patients, especially those who have discordantly low FVIII activity compared to von Willebrand factor (VWF) levels and who are suspected of having type 2N VWD, should include a FVIII binding assay (VWF:FVIIIB).

Grade B, level IIb [79,80,183]

See Evidence Table 5 [10] for additional information and detail.

3. Additional studies in selected persons may include the following:

a. Gene sequencing.

Grade C, level IV

b. Assays for antibodies to VWF.

Grade C, level IV

c. Platelet-binding studies.

Grade B, level III [177]

*Laboratory testing should be guided by the history and physical findings (Diagnostic Recommendations I) and results of the initial laboratory evaluation (II.A, above). For example, findings of liver disease may lead to a different or additional laboratory evaluation rather than an evaluation for VWD (II.B, above).

Diagnostic Recommendations III

III. Making the Diagnosis

A. *Clinical criteria.* These criteria include personal and/or family history and/or physical evidence of mucocutaneous bleeding. Until further validation of scoring systems and criteria for assessing bleeding history and the probability of von Willebrand disease (VWD), especially type 1 VWD, the Expert Panel suggests that an increasing number of positive responses to the questions about bleeding (Diagnostic Recommendations I; Fig. 3; Table 9) and abnormal findings on physical examination increase the likelihood that an individual has a bleeding disorder, including possible VWD.

AND

B. *Laboratory criteria.* The values in the following table represent prototypical cases without additional

Condition	VWF:RCo (IU dL^{-1})	VWF:Ag (IU dL^{-1})	FVIII	Ratio of VWF:RCo/VWF:Ag
Type 1	<30*	<30*	↓ or normal	>0.5 to 0.7
Type 2A	<30*	<30–200*†	↓ or normal	<0.5 to 0.7
Type 2B	<30*	<30–200*†	↓ or normal	Usually <0.5 to 0.7
Type 2M	<30*	<30–200*†	↓ or normal	<0.5 to 0.7
Type 2N	30–200	30–200	↓↓	>0.5 to 0.7
Type 3	<3	<3	↓↓↓ (<10 IU dL^{-1})	Not applicable
'Low VWF'	30–50	30–50	Normal	>0.5 to 0.7
Normal	50–200	50–200	Normal	>0.5 to 0.7

FVIII, factor VIII; VWD, von Willebrand disease; VWF, von Willebrand factor; VWF:Ag, von Willebrand factor antigen; VWF:RCo, von Willebrand factor ristocetin cofactor activity.

↓ refers to a decrease in the test result compared to the laboratory reference range.

*Less than 30 IU dL^{-1} is designated as the level for a definitive diagnosis of VWD; some patients with type 1 or type 2 VWD have levels of VWF:RCo and/or VWF:Ag of 30-50 IU dL^{-1}.

†The VWF:Ag in the majority of individuals with type 2A, 2B or 2M VWD is <50 IU dL^{-1}.

von Willebrand factor (VWF) (or other disease) abnormalities in the patient. In practice, exceptions occur, and repeated testing and clinical experience are important and may be necessary for interpretation of laboratory results.

1. Although published evidence is limited, for defining the ratio of VWF:RCo/VWF:Ag to use for distinguishing type 1 VWD versus type 2 VWD variants (A, B, or M), the Expert Panel recommends a ratio of less than 0.5–0.7 until more laboratories clearly define a reference range using large numbers of normal subjects and persons with type 1 VWD and type 2 VWD variants.
Grade C, level IV [72,73,93,158,184,185]

2. The Panel currently recommends that 30 IU dL^{-1} be used as the cutoff level for supporting the definite diagnosis of VWD for the following reasons:
• There is a high frequency of blood type O in the United States, and it is associated with 'low' VWF levels [45].
• Bleeding symptoms are reported by a significant proportion of normal individuals [134–136,148].
• No abnormality in the VWF gene has been identified in many individuals who have mildly to moderately low VWF:RCo levels.
Grade C, level IV [102–104]

This recommendation does not preclude the diagnosis of VWD in individuals who have VWF:RCo of 30–50 IU dL^{-1} if there is supporting clinical and/or family evidence for VWD. This recommendation also does not preclude the use of agents to increase VWF levels in those who have VWF:RCo of 30–50 IU dL^{-1} and may be at risk for bleeding.

Management of VWD

Introduction

Therapies to prevent or control bleeding in persons with VWD follow three general strategies. The first strategy is to increase the plasma concentration of VWF by releasing endogenous VWF stores through stimulation of endothelial cells with desmopressin. The second approach is to replace VWF by using human plasma-derived, viral-inactivated concentrates. The third strategy uses agents that promote haemostasis and wound healing but do not substantially alter the plasma concentration of VWF. The three treatment options are not mutually exclusive, and patients may receive any one or all three classes of agents at the same time. The appropriateness of therapeutic choice depends on the type and severity of VWD, the severity of the haemostatic challenge, and the nature of the actual or potential bleeding. Because some persons who have a VWF:RCo level higher than 30 IU dL^{-1} manifest clinical bleeding, persons not having a definite diagnosis of VWD but who have low VWF and a bleeding phenotype may merit treatment or prophylaxis of bleeding in certain clinical situations [191].

Infusions of VWF to prevent bleeding episodes — known as prophylaxis — are less frequently required in patients with severe VWD in contrast to patients with severe haemophilia. The Centers for Disease Control and Prevention Universal Data Collection Project Web site [207] reports that 45% of patients with severe haemophilia A use some type of prophylaxis, either continuous or intermittent, compared with 10% of patients with severe VWD. Risks and benefits of prophylaxis should be carefully weighed when considering long-term therapy for VWD [208,209].

Treatment of VWD in the United States varies widely and frequently is based on local experience and physician preference. Few standard recommendations exist to guide therapy for VWD [6,7,9]. This guideline document presents recommendations regarding the management and prevention of bleeding in persons with VWD and reviews the strength of evidence supporting those recommendations.

Non-replacement therapy with desmopressin to elevate VWF

Mechanism of action of desmopressin Desmopressin (DDAVP) is a synthetic derivative of the antidiuretic hormone, vasopressin. Desmopressin has been used to treat VWD for more than 25 years, and its pharmacology, mechanism of action and indications have been reviewed extensively [210–212]. Desmopressin stimulates the release of VWF from endothelial cells through its agonist effect on vasopressin V2 receptors [210,211,213]. The mechanism by which desmopressin increases plasma concentration of VWF is probably through cyclic adenosine monophosphate (cAMP)-mediated release of VWF from endothelial cell Weibel-Palade bodies [213,214]. FVIII levels also increase acutely after administration of desmopressin, although the FVIII storage compartment and the mechanism of release by desmopressin have not been fully elucidated [12,215]. Desmopressin induces the release of tissue plasminogen activator (tPA) [216,217]. However, the secreted tPA is rapidly inactivated by plasminogen activator inhibitor type 1 (PAI-1) and does not appear to promote fibrinolysis or bleeding after desmopressin treatment.

Table 12. Intravenous desmopressin effect on plasma concentrations of FVIII and VWF in normal persons and persons with VWD.

| Group/source | Number | Mean increase (fold)* | | | Type of evidence |
		VWF:RCo	VWF:Ag	FVIII	
Normals					
Mannucci *et al.* [218]	10	NA	3.3	4.5	Case series
Lethagen *et al.* [219]	10	NA	2.7	3.7	Case series
VWD					
Type 1					
Mannucci *et al.* [218]	15	NA	3.6	5.5	Case series
de la Fuente *et al.* [220]	13	5.0	4.5	5.7	Case series
Mannucci *et al.* [94]	7	9.5	9.0	10.3	Case series
Rodeghiero *et al.* [221]	14	NA	NA	7.8	Case series
Mannucci *et al.* [222]	15	5.1	4.8	4.3	Case series
Revel-Vilk *et al.* [223] – 91% response rate[†]	56	NA	NA	NA	Retrospective review
Federici *et al.* [224] – 27% response rate[†]	26	3.1	NA	3.3	Case series
Type 1, Vicenza (ultralarge multimers)					
Mannucci *et al.* [94]	6	9.1	8.3	10.2	Case series
Rodeghiero *et al.* [221]	5	NA	NA	10	Case series
Type 1, severe[‡]					
Revel-Vilk *et al.* [223] – 36% response rate[†]	14	NA	NA	NA	Retrospective review
Federici *et al.* [224] – 27% response rate[†]	26	3.1	NA	1.4	Case series
Type 1, severe with normal platelet VWF					
Rodeghiero *et al.* [225]	14	NA	NA	7.8	Case series
Mannucci *et al.* [101] – 6/6 with increase in FVIII, VWF:RCo and VWF:Ag	6	NA	NA	NA	Case series
Type 1, 'platelet low'					
Mannucci *et al.* [101] – 7/7 with increase in FVIII; 0/7 with increase in VWF:RCo or VWF:Ag	7	NA	NA	NA	Case series
Rodeghiero *et al.* [221] – 2/2 with increase in FVIII; VWF response not reported	2	NA	NA	2.1	Case series
Type 2A					
de la Fuente *et al.* [220] – 86% response rate[†]	7	6.4	4.9	4.9	Case series
Revel-Vilk *et al.* [223] – 40% response rate, results given for responders only	5	4.2	NA	2.9	Retrospective review
Federici *et al.* [224] – 7% response rate[†]	15	2.6	NA	3.4	Case series
Type 2B					
Casonato *et al.* [66]	4	2.3	3.5	3.0	Case series
McKeown *et al.* [226]	3	3.2	3.2	3.6	Case series
Castaman and Rodeghiero [227]	33	Normalized in 18/33	Normalized in 33/33	Normalized in 33/33	
Type 2M					
Federici *et al.* [224] – 14% response rate[†]	21	3.3	NA	3.0	Case series
Type 2N					
Mazurier *et al.* [228]	8	1.4	2.2	9.7	Case series
Federici *et al.* [224] – 75% response rate[†]	4	3.8	NA	6.6	Case series
Type 3					
Castaman *et al.* [229] – 0% response rate[†]	6	1.8	8.6	2.5	Case series

FVIII, factor VIII; NA, not available; VWD, von Willebrand disease; VWF, von Willebrand factor; VWF:Ag, von Willebrand factor antigen; VWF:RCo, von Willebrand factor ristocetin cofactor.
*Data are given as mean fold increase in plasma factor compared to baseline after a single administration of desmopressin. Mean fold increases were calculated from original data, where possible, if not included in the manuscript.
[†]Response defined as twofold increase AND to at least 30 IU dL^{-1} VWF:RCo and FVIII.
[‡]VWF:RCo <10 IU dL^{-1} OR bleeding time >15 min OR FVIII <20 IU dL^{-1}.

Desmopressin dosing and administration Table 12 displays published reports of desmopressin effects on laboratory assays of VWF and FVIII in normal persons and persons who have various subtypes of VWD [66,94,101,218–229]. When administered intravenously (i.v.) to normal persons as well as to patients with VWD or mild haemophilia, desmopressin consistently increases plasma VWF and FVIII

from twofold to more than fivefold over baseline levels [94,215,218–223]. Children younger than 2 years have a lower response rate than older children [223]. Two controlled prospective studies in healthy volunteers form the basis for desmopressin dosing recommendations [218,219]. Maximal FVIII response was determined at 0.3 µg kg^{-1} in both studies, while maximal VWF release data were determined at 0.2 and 0.3 µg kg^{-1} in the same two studies. On the basis of these data, standard dosing of desmopressin is 0.3 µg kg^{-1} given i.v. in 30–50 mL of normal saline over 30 min, with peak increments of FVIII and VWF 30–90 min after the infusion [215,219,220,230]. Nasal administration of high-dose desmopressin acetate (Stimate; CSL Behring, King of Prussia, PA, USA) is often effective for minor bleeding, but i.v. administration is the preferred route for prophylaxis of surgical bleeding and for treatment of major haemorrhage.

A retrospective review of desmopressin administration to 56 children who had non-severe type 1 VWD found a 91% response rate, defined as a twofold increase in FVIII and VWF activity, to at least 30 IU dL^{-1} [223]. In a small case series of VWD patients, the consistency of FVIII increments and the responses of the BT after a second test dose of desmopressin were within 10–20% of the initial values [221]. Evidence shows that response to desmopressin diminishes with repeated doses, probably because of depletion of the VWF storage compartment [215,218]. However, when desmopressin was given in four daily doses to 15 patients who had type 1 VWD, an increase in FVIII activity of at least twofold was found in 100% of the patients after the first administration, in 80% after the second, in 87% after the third and in 74% after the fourth administration [222].

Consistency of response to desmopressin has been studied using three to four once-daily doses [218,222]. A series of 15 type 1 VWD patients showed a mean increase of VWF:RCo to fivefold above baseline after the first dose of desmopressin, significantly decreased response to fourfold after the second daily dose, and no significant change in response between the second and third and third and fourth doses [222]. The proportion of VWD patients attaining at least a twofold increase in FVIII activity after the second to fourth daily doses – 80% after the second daily dose of desmopressin to 74% after the fourth dose – was substantially higher than that for haemophilia A patients (55% vs. 37%). There is no published evidence regarding response to desmopressin given every 12 h to compare with daily dosing of desmopressin. In addition to tachyphylaxis, hypona-traemia may complicate repeated desmopressin dosing, and fluid restriction and serum sodium monitoring are recommended.

Desmopressin can also be administered subcutaneously (s.c.) or intranasally [218,219,230]. The effective s.c. dose is identical to the i.v. dose, but the s.c. preparation is not available in the United States. The preparation of desmopressin for nasal instillation (Stimate) contains 150 µg of desmopressin per metered nasal puff (0.1 mL of a 1.5 mg mL^{-1} solution). The dose is one puff for persons who weigh <50 kg and two puffs (one puff to each nostril) for persons weighing 50 kg or more. Although the intrasubject and intersubject CV for reproducibility of nasal spray effect is good, nasal absorption is variable and all patients with VWD and who are responsive to i.v. desmopressin should undergo a trial of nasal desmopressin (Stimate) to measure FVIII and VWF response before using it [219]. When used for epistaxis, intranasal desmopressin ideally is delivered into the non-bleeding nostril. Persons who have inadequate plasma responses to i.v. desmopressin will not respond to intranasal desmopressin.

Another nasal formulation of desmopressin for enuresis contains 10 µg per puff (about 7% of the Stimate concentration); however, this preparation is not suitable for treatment of VWD. Patients and parents must be carefully instructed regarding the two concentrations of nasal desmopressin — the one used for bleeding (1.5 mg mL^{-1}) and the one used for antidiuretic hormone replacement (diabetes insipidus) and bedwetting (0.1 mg mL^{-1}) — to avoid accidental underdosing for VWD.

Monitoring of VWD patients receiving desmopressin Treatment of patients who have VWD with desmopressin should be based on results of a therapeutic trial, ideally one performed in patients in a non-bleeding state and before general clinical use. Although the pattern of desmopressin responsiveness is fairly consistent within VWF subtypes, population results should not be used to plan treatment of individual patients (Table 12). VWF:RCo and FVIII activities should be measured in all VWD patients at baseline and within 1 h after administering desmopressin. Additional assay of VWF:RCo and FVIII, done 2–4 h after desmopressin administration, will evaluate for shortened survival and should be considered for patients who have a history of poor response to treatment [43].

According to conservative definitions of laboratory response, the majority of patients with type 1 VWD respond adequately to desmopressin (Table 12).

Single infusions of desmopressin for common bleeding episodes — such as epistaxis, simple dental extraction or menorrhagia — do not usually require laboratory monitoring. Patients should be monitored for VWF:RCo activity as well as FVIII activity around major surgical procedures or major bleeding events. For major surgical procedures or bleeding events, patients with VWD should be referred to hospitals with in-house or daily laboratory availability of FVIII and VWF:RCo activity assays. Care should be taken to monitor serum electrolytes, especially after surgery or multiple doses of desmopressin. Adult patients, especially those who are elderly, should be evaluated for cardiovascular disease before using desmopressin because myocardial infarction rarely has been precipitated by desmopressin therapy in patients with haemophilia or uraemia [231–233].

Pharmacokinetics of VWF and FVIII after desmopressin After stimulation with desmopressin, released VWF and FVIII circulate with an apparent half-life characteristic of the patient's own proteins, or approximately 8–10 h for both proteins in normal individuals [215]. Type 2 VWF proteins that are released by desmopressin increase in concentration but retain their intrinsic molecular dysfunction [234]. For this reason, desmopressin has been efficacious in only a minority of patients with types 2A or 2M VWD. Therefore, monitoring is necessary to document adequate correction of VWF:RCo. Type 2N VWF lacks FVIII stabilization; consequently, patients with 2N VWF release FVIII and the abnormal VWF protein as expected, but the survival of released FVIII may be severely decreased, with an apparent plasma half-life as low as 2 h, depending on the mutation [163,228]. Emerging information suggests that some individuals with type 1 VWD have accelerated plasma clearance of VWF and may benefit from trial testing of VWF:RCo 2–4 h after a dose of desmopressin [42,235].

After infusion of desmopressin into patients with type 2B VWD, VWF multimers of larger but still somewhat less than normal molecular weight can be detected in plasma after 15–30 min, with persistence throughout 4 h of study [65,163,234,236]. Although formal pharmacokinetic studies have not been reported for type 2B VWD, VWF:RCo activity increases were less than that seen in type 1 VWD with an apparent half-life of approximately 4 h [65,226]. Bleeding time response to desmopressin in type 2B VWD is inconsistent [226,227].

Clinical response to desmopressin in VWD The clinical effectiveness of desmopressin to prevent or control bleeding depends, in large part, on the plasma VWF:RCo or FVIII activity achieved after drug administration, which in turn depends primarily on the basal levels of plasma FVIII and VWF:RCo and to a lesser extent on the underlying qualitative VWF defect [66,94,101,218–229]. Table 13 and Evidence Tables 7–11 [10] summarize published data on the clinical response when using desmopressin in conjunction with common surgical procedures [133,215,220,223,225,227,229,237–247]. All data were derived from retrospective studies and small case series; there are no randomized-clinical trials of the use of desmopressin in persons with VWD.

Table 13. Clinical results of desmopressin treatment in patients with von Willebrand disease*.

Surgical prophylaxis	Number	Frequency	Duration	Other treatment	Bleeding outcome
Adults					
Dental procedures [65,66,220,225,227,229,237–241,380,396]	113	Once	Once or twice	Antifibrinolytics	Excellent/good in 109/113
Gynaecological [215,220,238,239]	9	Daily	1–7 days	Antifibrinolytics 2/7	Delayed bleeding in 2/9 requiring extended desmopressin treatment for 3 and 6 days
Surgery [215,220,229,239,242,255,380]	26	Daily	1–5 days		Excellent/good in 25/26 Haemorrhage after 1 rhinoplasty
Children					
Primarily tonsillectomy and adenoidectomy [241–247]	146	Once or twice daily	1–7 days	Antifibrinolytics in most for 7 days	Excellent/good in 125/146
Primarily tonsillectomy/adenoidectomy [261]	119	NA	NA	NA	Excellent/good in 105/119
Otologic surgery [242]	6	Daily	2 days		Excellent/good in 6/6

NA, not available.
*For additional detail and information, see Evidence Tables 7–11 [10].

Whether desmopressin will be adequate for prophylaxis around surgery or for treatment of bleeding events in persons with type 1 VWD depends on the severity of the haemostatic challenge and the time required for healing. Major surgery requires haemostasis for 7–14 days [239,248–253], whereas minor surgical procedures can be treated adequately in 1–5 days [239,245,246,253]. If treatment is necessary for more than 3 days, VWF concentrate is usually given to supplement therapy with desmopressin [239,246]. Currently, however, expert opinions are divided regarding the risk of delayed haemorrhage 5–10 days after a bleeding challenge in VWD patients, e.g. those who have had tonsillectomy or given birth. In small case series, persons with type 1 VWD Vicenza manifested an exaggerated response to desmopressin [43,94,221]. Individuals with type 2N VWD exhibit a brisk rise in plasma FVIII after receiving desmopressin, but they have a mean FVIII half-life of only 3 h because of deficient FVIII stabilization by the defective VWF [228]. Persons with low-platelet VWF or type 2A VWD have a low likelihood of having a clinically relevant desmopressin response, but they may warrant a desmopressin trial [221,224]. Type 2B VWD previously was a contraindication to desmopressin therapy because platelet counts usually fell after desmopressin stimulation [254]. However, thrombocytopenia after desmopressin in type 2B VWD is usually transient and often is not associated with bleeding or thrombosis [255]. In patients with type 2B VWD, decrease in platelet count after desmopressin administration has been considered 'pseudothrombocytopenia' by some authors because it is related to platelet agglutination *in vitro* rather than *in vivo* agglutination and clearance [65,236]. Therefore, desmopressin may be cautiously considered for patients with type 2B VWD. Patients with type 3 VWD almost never experience a clinically relevant rise in VWF:RCo or FVIII activities, and desmopressin is not considered clinically useful in these patients [223,224].

Complications and toxic effects of desmopressin Minor adverse effects of desmopressin are common and include facial flushing, transient hypertension or hypotension, headache or gastrointestinal upset [211,212,256], but these effects rarely limit clinical use. Water retention after a dose of desmopressin, with an increase in urinary osmolality, is universal; however, decreased serum sodium in otherwise healthy adults is variable and is related to multiple doses [256,257]. In the case of repeated dosing, all patients should be instructed to limit fluid intake to maintenance levels for 24 h [258–260]. Prophylactic use of desmopressin complicates the management of fluids and electrolytes for surgery or during childbirth. Seizures have been associated with hyponatraemia after desmopressin administration, primarily in young children [257,260]. Most paediatric haematologists do not use desmopressin in children <2 years old [223,260,261].

Myocardial infarction after treatment with desmopressin has been reported, although rarely, in patients with mild haemophilia A [231,233,262]. Desmopressin should be avoided in patients who are at very high risk for cardiovascular or cerebrovascular disease, especially the elderly, as underlying inhibition of plasminogen activation with desmopressin-related vasoconstriction contributes additional prothrombotic effects in these patients [263]. Because of reported complications in other patient populations, desmopressin should be used with caution for brain, ocular and coronary artery surgeries [232,264,265], and VWF concentrate replacement generally is used in these settings. Desmopressin does not appear to increase myometrial contractility greatly; consequently, pregnancy is not an absolute contraindication [266–269] but use of desmopressin is rarely indicated (see Pregnancy).

Replacement therapies to elevate VWF concentration: VWF concentrates

As of January 2007, Humate-P (CSL Behring) and Alphanate SD/HT (Grifols USA, Los Angeles, CA, USA) are plasma-derived concentrates licensed in the United States to replace VWF in persons who have VWD. One other plasma derivative — Koate DVI (Talecris Biotherapeutics USA, Research Triangle Park, NC, USA) — is licensed in the United States to treat haemophilia and has been used off-label for VWD. These products are not identical, having differing ratios of FVIII to VWF, and should not be considered as interchangeable [270–272]. All these products are manufactured at US-licensed facilities from pooled plasma collected from paid donors.

Products that contain FVIII and little or no VWF are generally not useful to treat VWD, but in rare circumstances these products may be used to treat patients who have antibody-mediated AVWS [273]. These products include the plasma-derived concentrates Monoclate P (CSL Behring), Monarc-M (Blood Diagnostics, Inc., Irmo, SC, USA) and Hemofil M (Baxter Healthcare Corp., Deerfield, IL, USA) and recombinant products Helixate FS (CSL Behring), Kogenate FS (Bayer HealthCare, Berkeley, CA, USA), Recombinate (Baxter Healthcare), Advate

(Baxter Healthcare) and ReFacto (Wyeth Pharmaceuticals, Philadelphia, PA, USA).

Humate-P, a lyophilized concentrate of purified VWF and FVIII, contains other plasma proteins, including fibrinogen and albumin. In Humate-P, the quantity of the large, most haemostatically active multimers of VWF is decreased compared to fresh plasma [274]. When reconstituted at the recommended volume, each millilitre of the product contains 50–100 IU mL^{-1} VWF:RCo and 20–40 IU mL^{-1} FVIII activity [275]. The median half-life of VWF:RCo activity was 10.3 h (range: 6.4–13.3) in one study [275] and 11.3 h in another study [248]. The product is indicated for use in adult and paediatric patients for treatment of spontaneous and trauma-induced bleeding when use of DDAVP is thought or known to be inadequate or contraindicated. Humate-P has received FDA approval for use in prophylactic management of surgery and invasive procedures in patients with VWD.

Alphanate SD/HT is a lyophilized concentrate of VWF and FVIII and other plasma proteins. It is prepared from pooled human plasma by cryoprecipitation of FVIII, fractional solubilization and further purification using heparin-coupled, cross-linked agarose. Upon reconstitution to the recommended volume, each millilitre of product contains 40–180 IU mL^{-1} FVIII activity and not less than 16 IU mL^{-1} VWF:RCo activity [276]. The median half-life for VWF:RCo activity was 6.91 h (mean, 7.46 ± 3.20 h; range: 3.68–16.22) [276]. The product is indicated for bleeding prophylaxis in patients with VWD, except type 3, who are undergoing surgical or invasive procedures and in whom desmopressin (DDAVP) is either ineffective or contraindicated.

Adverse reactions to VWF concentrates are rare but include allergic and anaphylactic symptoms, urticaria, chest tightness, rash, pruritus and oedema [250]. If these reactions occur, the infusion should be stopped, and appropriate treatment should be given as required. These products should be used with caution in patients who have known risk factors for thrombosis, as there have been a few reports of venous thromboembolism associated with high levels of FVIII [277,278]. Risk factors include old age, previous thrombosis, obesity, surgery, immobility, oestrogen therapy and use of antifibrinolytic therapy. If patients receive VWF replacement therapy continuously for several days, it has been recommended that FVIII levels be monitored to avoid unacceptably high levels [212,278].

Each of the products that contains VWF:RCo activity differs significantly in their ratios of VWF:RCo to FVIII [249,279], and the dose or frequency of dosing should not be assumed to be the same for all. The ratio of VWF:RCo to FVIII for Humate-P in various reports is 2.7, 2 and 1.6; for Koate-DVI, the ratio is 1.2 and 0.8 and for Alphanate, the ratio is 0.5. These products also differ in their relative levels of high-molecular-weight multimers. Koate-DVI, in particular, has fewer large VWF multimers compared to Alphanate SD/HT, which has fewer than Humate-P or normal plasma [272,281,282]. Recombinant VWF has been prepared and evaluated in animal models [283] but is not available for use in humans.

Cryoprecipitate, derived from plasma, historically has been used to treat haemophilia A and VWD. Although cryoprecipitate is not required to have a specified level of VWF, the final product must have on average at least 80 units of FVIII per standard donor unit [284]. Currently, cryoprecipitate is used under rare circumstances to treat VWD, such as when potential exposure to infectious agents can be limited by using directed donations to prepare the product [285]. However, the use of cryoprecipitate is strongly discouraged by the National Hemophilia Foundation, except in life- or limb-threatening situations when no VWF concentrate is available, because cryoprecipitate is not virally inactivated [286]. In developing countries, patients with VWD may have no other options, because virally inactivated plasma concentrates are not available or are too expensive [280], but use of cryoprecipitate poses a serious risk of disease transmission [287].

VWF concentrates are dosed primarily on the basis of labelled VWF:RCo units and secondarily on the basis of labelled FVIII units. A dosing trial with pharmacokinetic laboratory monitoring should be considered before major surgery for selected patients with type 3 VWD or AVWS who are at risk for poor VWF recovery because of inhibitors. Use of VWF concentrates to prevent or control bleeding has been clinically efficacious, as shown in Table 14. The ultimate goal of surgical prophylaxis is to achieve a therapeutic level of 100 IU dL^{-1} of VWF:RCo and, at least for the first 3 days of treatment, a nadir of 50 IU dL^{-1} of VWF:RCo, as well as similar targets for FVIII [248,250–253,288]. Successful surgical haemostasis was reported with the use of continuous infusion after initial bolus infusion at rates of 1–2 U kg^{-1} per hour of VWF:RCo [249].

Replacement therapy, using a VWF concentrate, is indicated for severe bleeding events or major surgery in patients with types 2 and 3 VWD as well as in patients with type 1 VWD and who are unresponsive to desmopressin or require protracted therapy, or

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Table 14. Efficacy of VWF replacement concentrate for surgery and major bleeding events*.

Source	Number	Uses	Loading dose[†] (U kg^{-1})	Outcome
Michiels et al. [252]	5	Surgery	60–80	100% Excellent–good
Thompson et al. [253]	42	Surgery	82.3	100% Excellent–good
Gill et al. [403]	53	Bleeding events	67	98% Excellent–good
Lillicrap et al. [250]	344/73	Bleeding events/surgery	55.3/69.1	99% Excellent–good
Nitu-Whalley et al. [239]	10	Surgery	54	100% Excellent–good
Lubetsky et al. [251]	3/9	Bleeding events/surgery	39.5	92.5% Excellent–good
Dobrkovska et al. [248]	73	Surgery	80[†]	99% Excellent–good
Hanna et al. [249]	5	Surgery	25–100[‡§]	100% Excellent–good
Kreuz et al. [246]	26/41	Bleeding events/surgery	10–50[§]	100% Excellent–good
Scharrer et al. [288]	66/70	Bleeding events/surgery	20–80[§]	100% Excellent–good

FVIII, factor VIII; VWF, von Willebrand factor; VWF:RCo, von Willebrand factor ristocetin cofactor activity.
*For additional details and information, see Evidence Table 12 [10].
[†]Loading dose (VWF:RCo IU dL^{-1}) reported as median except for Lubetsky et al. [251] (mean).
[‡]Continuous infusion was used after the loading dose.
[§]Loading dose (FVIII IU dL^{-1}).

Table 15. Suggested durations of VWF replacement for different types of surgical procedures.

Major surgery, 7–14 days*
    Cardiothoracic
    Caesarean delivery
    Craniotomy
    Hysterectomy
    Open cholecystectomy
    Prostatectomy
Minor surgery, 1–5 days*
    Biopsy: breast, cervical
    Complicated dental extractions
    Gingival surgery
    Central line placement
    Laparoscopic procedures
Other procedures, if uncomplicated, single VWF treatment
    Cardiac catherization
    Cataract surgery
    Endoscopy (without biopsy)
    Liver biopsy
    Lacerations
    Simple dental extractions

VWD, von Willebrand disease; VWF, von Willebrand factor.
*Individual cases may need a longer or shorter duration depending on the severity of VWD and the type of procedure.

Table 16. Initial dosing recommendations for VWF concentrate replacement for prevention or management of bleeding.

Major surgery/bleeding
Loading dose*	40–60 U kg^{-1}
Maintenance dose	20–40 U kg^{-1} every 8–24 h
Monitoring	VWF:RCo and FVIII trough and peak, at least daily
Therapeutic goal	Trough VWF:RCo and FVIII >50 IU dL^{-1} for 7–14 days
Safety parameter	Do not exceed VWF:RCo 200 IU dL^{-1} or FVIII 250–300 IU dL^{-1}

May alternate with desmopressin for latter part of treatment
Minor surgery/bleeding
Loading dose*	30–60 U kg^{-1}
Maintenance dose	20–40 U kg^{-1} every 12–48 h
Monitoring	VWF:RCo and FVIII trough and peak, at least once
Therapeutic goal	Trough VWF:RCo and FVIII >50 IU dL^{-1} for 3–5 days
Safety parameter	Do not exceed VWF:RCo 200 IU dL^{-1} or FVIII 250–300 IU dL^{-1}

May alternate with desmopressin for latter part of treatment

FVIII, factor VIII; VWF, von Willebrand factor; VWF:RCo, von Willebrand factor ristocetin cofactor activity.
*Loading dose is in VWF:RCo IU dL^{-1}.

where desmopressin is contraindicated. The dose and duration of therapy depend on the haemostatic challenge and expected duration required for haemostasis and wound healing. Major surgery requires haemostasis for 7–14 days [239,248–253], whereas minor surgical procedures can be treated adequately in 1–5 days [239,245,246,253]. Certain procedures can be managed adequately by using a single infusion of 20–40 U kg^{-1} of VWF:RCo before the procedure. Table 15 lists examples of major and minor surgical procedures. Table 16 lists initial dosing recommendations for use of VWF replacement therapy to prevent or treat bleeding. These recommendations are based on published results (Table 14) as well as consensus expert opinion.

The adequacy of courses of VWF replacement usually should be confirmed by laboratory assessment of VWF:RCo and FVIII levels, although monitoring of single infusions for treatment of outpatients may not be necessary. Duration of VWF elevation after replacement therapy is highly variable in the surgical setting. Thromboembolic events have been reported in patients with VWD and who are in situations of high thrombotic risk and receiving VWF:RCo/FVIII complex replacement therapy, especially in the setting

of known risk factors for thrombosis [277–279]. In all patients who have VWD and are receiving VWF concentrate, attention should be given to avoid exceeding maximal recommended levels of VWF:RCo and FVIII, perform proper thrombotic-risk assessment, and institute appropriate preventive strategies.

Human platelets contain 10–15% of total blood VWF, and platelet transfusions have been used successfully to treat bleeding in VWD patients [289,290]. Platelet transfusion therapy should be considered as an adjunctive source of VWF, especially in patients with type 3 or platelet-low VWD and PLT-VWD, to control bleeding that is non-responsive or poorly responsive to replacement therapy with VWF concentrate.

Other therapies for VWD

Antifibrinolytics The antifibrinolytic drugs amino-caproic acid and tranexamic acid are agents that inhibit the conversion of plasminogen to plasmin, inhibiting fibrinolysis and thereby helping to stabilize clots that have formed. Studies in haemophilia and in prostatectomy provided the basis for initial trials of antifibrinolytic agents in VWD [291]. The drugs can be used orally or i.v. to treat mild mucocutaneous bleeding in patients with VWD. In patients with mild-to-moderate VWD, tranexamic acid given topically in the oral cavity ('swish and swallow or spit') every 6 h has been used for prophylaxis in dental surgery, in combination with applied pressure, other topical agents and suturing of surgical sites [237]. The evidence for the effectiveness of local application of these agents is based on clinical case series [292], but this route of administration is not currently FDA approved. When desmopressin or VWF/FVIII concentrates are indicated, the use of antifibrinolytic agents as adjuncts to desmopressin or VWF concentrates has been helpful in controlling bleeding, such as in the oral cavity [133,220,225,229,237–240] and in the gastrointestinal and genitourinary tracts.

The usual adult dose of aminocaproic acid is 4–5 g as a loading dose orally or i.v. (1 h before invasive procedures), and then 1 g h^{-1}, i.v. or orally, or 4–6 g every 4–6 h orally, until bleeding is controlled, or for 5–7 days postoperatively [212]. Total daily dose of aminocaproic acid is limited to 24 g per 24 h to minimize potential adverse effects. Weight-based dosing is required in children and can also be used in adults (50–60 mg kg^{-1}) [212,292]. Lower doses (25 mg kg^{-1}) may be effective and can be used when gastrointestinal tract adverse effects interfere with therapy. Tranexamic acid is given i.v. at a dose of 10 mg kg^{-1} every 8 h [212]. The oral form is not currently available in the United States, and use of the i.v. form as an oral rinse ('swish and swallow' approach) is not an FDA-approved indication. The package insert for each drug should be consulted for more detailed guidance and for a full list of risks and contraindications. Both drugs can cause nausea and vomiting; less frequent but serious adverse effects include thrombotic complications. Both drugs are excreted renally, and dose adjustment or avoidance is advisable when significant renal insufficiency is present. Disseminated intravascular coagulation and bleeding from the renal parenchyma or upper urinary tract are relative contraindications to anti-fibrinolytic agents. Renovascular thrombi have followed use of antifibrinolytic agents in patients with disseminated intravascular coagulation and have caused renal failure. Patients have also experienced urinary tract obstruction with upper urinary tract bleeding, related to large clots in the renal pelvis or lower urinary tract. Changes in colour vision during therapy with tranexamic acid require cessation of the drug and ophthalmologic examination.

Topical agents Topical bovine thrombin (Thrombin-JMI; King Pharmaceuticals, Bristol, TN, USA) is marketed in the United States as an aid to haemostasis for topical therapy of accessible minor bleeding from capillaries and small venules. Fibrin sealant [Tisseel VH (Baxter), consisting of human thrombin, fibrinogen concentrate and bovine aprotinin] is indicated as an adjunct to haemostasis in certain surgical situations, but it is not effective for the treatment of massive and brisk arterial bleeding. Fibrin sealants have been used with good results as adjunctive therapy for dental surgery in persons with haemophilia or VWD [237,293]. Topical collagen sponges are also approved for control of bleeding wounds [294]. Other topical agents approved for limited indications include Coseal (Baxter), BioGlue (CryoLife, Inc., Kennesaw, GA, USA) and QuikClot (Z-Medica Corporation, Wallingford, CT, USA); however, no reports of their use in treating VWD could be found. QuikClot, containing the mineral zeolite, was approved recently by the FDA for use with compression dressings for control of external traumatic bleeding in the prehospital setting (e.g. on the battlefield). The added benefit of topical agents – when used with single or combination therapies, including antifibrinolytic drugs, desmopressin and VWF/FVIII concentrate – is unproven. The topical use of plasma-derived bovine or human proteins imparts a theoretical risk of disease transmission and of potential allergic and other immune reactions. The use of fibrin sealants in addition to drugs, concentrates or both may be viewed

as optional adjunctive therapy for dental surgery and for cases in which surface wound bleeding continues despite combined therapy with drugs and concentrates. The safety of these topical agents in therapy for VWD remains to be demonstrated.

Other issues in medical management

All persons with significant bleeding symptoms related to VWD are likely to require human blood product administration and should receive immunizations for hepatitis A and B as recommended for individuals with haemophilia [295]. Persons with VWD should be counselled to avoid aspirin, NSAIDs and other platelet-inhibiting drugs [296–298].

Treatment of AVWS

In an international registry of 189 cases of AVWS, desmopressin produced clinical and laboratory improvement in one-third of cases, although this effect was often short lived [114]. If FVIII activity and the PTT were abnormal, a good desmopressin response was less common than in hereditary VWD and was often brief. In the international registry series, most patients who had AVWS also received VWF/FVIII concentrates; the extent and duration of response varied. Therefore, VWF:RCo and FVIII levels must be measured preinfusion and postinfusion of desmopressin or VWF/FVIII concentrates in patients with AVWS to determine the extent and duration of response and to guide subsequent dosage and dosing intervals [114,152].

In patients who had a previous inadequate response to desmopressin and VWF/FVIII concentrates, i.v. immunoglobulin G (IGIV), 1 g kg^{-1} daily for 2 days, given alone was effective in controlling bleeding and raising VWF:RCo activity for 3 weeks in all eight patients who had excessive bleeding and an IgG monoclonal gammopathy of uncertain significance (MGUS) [299]. In the international registry series, one-third of the 63 patients treated with high-dose IGIV had a good response [114]. The underlying diagnoses of the responders were lymphoproliferative disorders (including MGUS), solid tumours and autoimmune diseases. An anti-VWF antibody could be demonstrated *in vitro* in about two-thirds of those responders. High-dose IGIV therapy in the setting of AVWS is an off-label use but should be considered when desmopressin and VWF/FVIII concentrate therapy fail to control bleeding symptoms adequately [300–302]. Some patients with immune-mediated AVWS have responded to plasmapheresis, corticosteroids and immunosuppressive agents [114]. Because many patients in the international registry series received multiple therapeutic modalities, the independent contribution of each therapy to clinical improvement was unclear.

When all other therapeutic modalities fail to control bleeding adequately, the infusion of recombinant activated factor VII (FVIIa) may be considered, but this agent should be used with caution. Little experience has been reported for its use in treating VWD. A recent report described acute myocardial infarction immediately after the second dose of 90 μg kg^{-1} in a 50-year-old man who had hereditary type 2A VWD, gastrointestinal tract bleeding and several risk factors for, but no history of, coronary artery disease [303].

Cardiac valvular diseases Congenital or acquired heart disease has been associated with AVWS [114,116,304]. Elevated shear stress around a stenotic valve or septal defect may promote the proteolysis and depletion of high-molecular-weight VWF multimers [131]. Patients who had associated aortic stenosis or other cardiac valvular disorders infrequently responded to any of the therapies described above [114,119]. After surgical correction of the cardiac defect, the multimer pattern has improved at least transiently in most patients studied [116,119,304]. Administration of VWF/FVIII concentrate immediately preoperatively should be considered for patients who demonstrate transient improvement in VWF activity with a test dose.

Angiodysplasia Bleeding from gastrointestinal angiodysplasia has been reported in persons with AVWS [305] as well as in persons with various types of congenital VWD. For example, bleeding from angiodysplasia is a classic presentation of AVWS associated with aortic stenosis [119,306] and is often resistant to medical therapy, requiring surgical correction of the valve defect to ameliorate bleeding symptoms. In the absence of a correctable underlying aetiology of angiodysplasia and bleeding associated with AVWS or congenital VWD, management of the condition can be challenging, as no single treatment modality is successful in all cases [307].

Thrombocytosis Thrombocytosis, especially in persons with essential thrombocythemia, is associated with a relative reduction in the proportion of high-molecular-weight multimers [122]. Although the relation of this abnormality to bleeding is inconsistent, treatment is aimed at reduction of the platelet count.

Hypothyroidism In contrast to the above syndromes, AVWS that occurs in hypothyroidism is caused by decreased synthesis, and the VWF multimer patterns are normal [308,309]. A minority of patients with hypothyroidism have VWF levels below normal, and not all who have low VWF levels have bleeding symptoms. The decrease in VWF is corrected by thyroid hormone replacement [124,309].

Management of menorrhagia in women with VWD

Menorrhagia is often the primary bleeding symptom in women with VWD [87,310,311]. Menorrhagia, however, may be a sign of a gynaecological disorder rather than VWD [312]. Therefore, a full gynaecological evaluation is required before therapy is initiated [312].

Medical therapies that have been described to control menorrhagia in women with VWD include combined oral contraceptives, tranexamic acid, desmopressin and, most recently, the levonorgestrel-releasing intrauterine system (Table 17). Data regarding the effectiveness of these therapies are limited. The only published randomized-clinical trial of desmopressin for menorrhagia was small and failed to demonstrate efficacy compared with placebo [313]. The available data show no evidence that desmopressin is more effective than other therapies used to treat menorrhagia [314]. Depending on the woman's age, underlying gynaecological condition and reproductive plans, any of the therapies demonstrated to be effective for the treatment of menorrhagia in women without VWD may be suitable, with the exception of NSAIDs, which decrease platelet function and systemic haemostasis [315]. In one retrospective review of 36 adolescent girls with VWD and menorrhagia, treatment using oral contraceptive pills or intranasal desmopressin were equally efficacious [316].

In adolescent or adult women who do not desire pregnancy, but may desire future child-bearing, the first choice of therapy should be combined oral contraceptives. Combined oral contraceptives contain a synthetic oestrogen (ethinyl estradiol) and a progestin [317]. The progestin prevents ovulation, and the synthetic oestrogen prevents breakthrough bleeding [318]. A majority of studies have found that combined oral contraceptives increase fibrinogen, prothrombin, FVII, FVIII and/or VWF [319–321] and, consequently, promote haemostasis. It is not known whether the increase in coagulation factors associated with combined oral contraceptives contributes to the clinical response, but combined oral contraceptives do reduce menstrual blood loss [322] and increase haemoglobin concentrations in women with anaemia [323–325]. Combined oral contracep-

Table 17. Effectiveness of medical therapy for menorrhagia in women with VWD.

Source	Population	Treatment	Controls	Results	Setting
Kouides *et al.* [367]	41 women known to have type 1 VWD	Oral contraceptives	No	76% 'ineffective'	Patient survey
Foster [339]	25 women known to have VWD	Oral contraceptives	No	88% 'effective'	Survey of haemophilia treatment centres
Greer *et al.* [329]	7 women with VWD	Oral contraceptives	No	3 'good effect' 3 'slight improvement'	Retrospective review, single haemophilia treatment centre
Kingman *et al.* [404]	13 women with VWD	Levonorgestrel intrauterine system	No	Periods 'much better' in 100%; 50% developed amenorrhea; increased haemoglobin concentrations	Prospective, open label
Kadir *et al.* [313]	39 women known to have VWD	Desmopressin	Placebo	No significant difference	Prospective, double-blind, randomized trial
Leissinger *et al.* [405]	90 women with various bleeding disorders	Desmopressin	No	92% 'excellent' or 'good'	Prospective, open label
Rodeghiero *et al.* [406]	43 women with VWD	Desmopressin	No	65% 'very effective'; 21% effective; 14% not effective	Prospective, open label
Mohri [407]	3 women with VWD	Tranexamic acid	No	3 'well-controlled'	Case series
Greer *et al.* [329]	2 women with VWD	Tranexamic acid	No	2 'no effect'	Retrospective review, single haemophilia treatment centre

VWD, von Willebrand disease.

tives, used by millions of women for prolonged periods of time, have been proven to be safe for long-term use [326] except in women with thrombophilia [318]. Although no formal studies of the effects of the contraceptive patch on haemostasis have been performed, the patch likely has effects similar to those of combined oral contraceptives [327].

For a woman who has VWD and would otherwise be a suitable candidate for an intrauterine device, the second choice of therapy should be the levonorgestrel-releasing intrauterine system. The levonorgestrel-releasing intrauterine system is a progestin-impregnated intrauterine device that is believed to reduce menstrual blood loss by opposing oestrogen-induced growth of the endometrium or lining of the uterus [328].

Women who do not respond to hormonal therapy and are being considered for treatment with desmopressin or VWF concentrate should be referred to a haemophilia treatment centre or to a haematologist who has expertise in haemostasis. Treatments specific for VWD (such as desmopressin or VWF concentrate) or antifibrinolytic therapy, although they have not been proven to be effective for menorrhagia, may be tried.

In addition to medical therapies, surgical therapies have been used to treat menorrhagia in women with VWD. Dilation and curettage (D&C), while occasionally necessary to diagnose intrauterine pathology, is not effective in controlling heavy menstrual bleeding [329]. In two cases reported by Greer *et al.* [329] and two cases reported by Kadir *et al.* [330], D&C resulted in further blood loss. Endometrial ablation, however, reduced menstrual blood loss in seven of seven women who had VWD [331]. Three of these seven women ultimately required hysterectomy, compared with the 12–34% of women without VWD who usually require hysterectomy [332–336]. Women with VWD and who undergo hysterectomy may be at greater risk of perioperative bleeding complications than other women, and bleeding may occur despite prophylactic therapy [134,311]. Hysterectomy carries the risk of bleeding complications, but women who require the operation should not be deprived of its benefits. Because menorrhagia is often the primary bleeding symptom experienced by a woman who has VWD, hysterectomy offers the possibility of the elimination of menorrhagia bleeding symptoms and improved quality of life [336–338].

Haemorrhagic ovarian cysts

Multiple case reports describe women who have VWD and have experienced haemorrhagic ovarian cysts [329,339–344]. Silwer [134], for example, reported that nine of 136 women (6.8%) who had VWD experienced this problem. Ovulation is not normally accompanied by any clinically significant bleeding, but in a woman who has a congenital bleeding disorder such as VWD, the potential exists for bleeding into the peritoneal cavity or bleeding into the residual follicle, resulting in a haemorrhagic ovarian cyst [343] or retroperitoneal haematoma [329]. Acute treatment of haemorrhagic ovarian cysts with surgical therapy, tranexamic acid and factor replacement has been reported [329,342,343]. Oral contraceptives have been used to prevent recurrences [340,341,343].

Pregnancy

Few options are available for the management of menorrhagia or recurrent haemorrhagic ovarian cysts in women who have VWD and desire pregnancy. Although data are limited to case reports, desmopressin, antifibrinolytics or VWF concentrate may be tried [339].

Ideally, planning for pregnancy begins before conception. Women who have VWD and are contemplating a pregnancy should be aware that they may be at increased risk of bleeding complications during pregnancy [345] and are definitely at increased risk of postpartum haemorrhage [139]. Before conception or during pregnancy, women should be offered the opportunity to speak with a genetic counsellor regarding the inheritance of VWD [346] and with a paediatric haematologist regarding the evaluation of the infant after delivery.

Women who have type 1, type 2 or type 3 VWD and have FVIII levels <50 IU dL^{-1}, VWF:RCo <50 IU dL^{-1} or a history of severe bleeding should be referred for prenatal care and delivery to a centre that, in addition to specialists in high-risk obstetrics, has a haemophilia treatment centre, a haematologist with expertise in haemostasis or both. Laboratory, pharmacy and blood bank support is essential. Before any invasive procedure, such as chorionic villus sampling, amniocentesis or cervical cerclage, women with VWD should have laboratory assays for FVIII and VWF:RCo to receive appropriate prophylaxis [346,347]. FVIII and VWF:RCo levels should be obtained in the third trimester of pregnancy to facilitate planning for the delivery [347]. Before delivery, all women with VWD should meet with an anaesthesiologist to plan for the possible need for the administration of haemostatic agents or alternatives, if necessary, for regional anaesthesia at the time of delivery [346]. A pregnant woman carrying a baby at

risk for type 3 VWD or severe forms of types 1 or 2 VWD should be referred to a paediatric haematologist for counselling regarding neonatal testing and potential perinatal bleeding complications in affected infants [87,345,348].

Data are limited on the use of desmopressin for VWD in pregnancy. Mannucci [349] reported using desmopressin for prophylaxis before procedures in 31 pregnant women 'without mishap', but specific data were not provided. Desmopressin, in the lower doses used to treat diabetes insipidus, however, is generally thought to be safe for mother and foetus. In a review of 53 women who were pregnant and used desmopressin, administered in doses of 7.5–100 μg day^{-1} for diabetes insipidus, no adverse maternal or neonatal effects were attributable to the medication [269]. In an *in vitro* placenta model, desmopressin did not cross the placenta in detectable amounts [269].

Tranexamic acid crosses the placenta [350] but has been used to treat bleeding during pregnancy in a limited number of cases without adverse foetal effects [351–356]. Data regarding aminocaproic acid in pregnancy are limited, but aminocaproic acid was not found to be teratogenic in rabbits [357]. In cases of its use during pregnancy, no adverse foetal effects have been reported [358].

Miscarriage and bleeding during pregnancy In the general population, miscarriage is common, and 12–13.5% of diagnosed pregnancies result in spontaneous abortion [359,360]. Although detailed data were not provided, in a study of 182 women who had severe VWD, Lak *et al.* [87] reported that miscarriage was no more frequent than in the general population. Other studies, however, have found a higher incidence of miscarriage among women with VWD than in controls [139] or in the background rate [339,345].

Bleeding complications during pregnancy other than miscarriage have been reported [329,339,361–363]. Kadir *et al.* [345] found that 33% of women who had VWD bled during their first trimester.

Childbirth Table 18 summarizes nine case series reporting pregnancy outcomes in women with VWD, including rates of miscarriage, peripartum prophylaxis, postpartum haemorrhage and perineal haematoma. Prophylaxis included cryoprecipitate, fresh-frozen plasma, desmopressin and factor replacement.

No large prospective studies correlate VWF:RCo and FVIII levels with the risk of bleeding at the time of childbirth, but the opinion of experts is that VWF:RCo and FVIII levels of 50 IU dL^{-1} should be achieved before delivery [329] and maintained for at least 3–5 days afterward [9,212,329,345–347]. There is no consensus on levels of VWF:RCo and FVIII that are safe for regional anaesthesia [364], but if VWF:RCo and FVIII levels are 50 IU dL^{-1} or higher and the coagulation screen is normal, regional anaesthesia may be considered safe [345].

Desmopressin may be used to raise factor levels in responders, but care must be taken in its administration at the time of childbirth. Women commonly receive 1–2 L or more of fluid at the time of a vaginal delivery and 2–3 L or more at the time of caesarean delivery. Fluids containing oxytocin, which also causes fluid retention, combined with desmopressin may result in fluid retention and life-threatening hyponatraemia. Chediak and colleagues [363] reported complications of fluid retention in two women who received desmopressin at the time of childbirth. One woman who received three doses 18 h apart developed severe hyponatraemia (sodium level of 108 mEq L^{-1}) and experienced grand mal seizures.

Because NSAIDs, commonly prescribed for pain after childbirth, may decrease platelet function and systemic haemostasis [315], alternative analgesics should be considered.

Postpartum haemorrhage Postpartum haemorrhage is an anticipated problem among women with VWD. By the end of gestation, an estimated 10–20% of a woman's blood volume, or at least 750 mL min^{-1}, flows through the uterus [365]. After delivery of the infant and placenta, the uterus must contract, and the uterine vasculature must constrict to prevent exsanguination [366]. Failure of the uterus to contract is the single most important cause of postpartum haemorrhage [366]. Nonetheless, women with VWD have a greater risk of postpartum haemorrhage than controls [134,139,367]. Multiple case series document an increased incidence of postpartum haemorrhage in women with VWD (Table 18).

Perineal haematoma, a rare complication of vaginal birth, occurs with some frequency in women with VWD. Greer *et al.* [329] reported one haematoma in 13 vaginal deliveries, and Kadir and colleagues [345] reported three haematomas in 49 vaginal deliveries. This is a relatively high frequency compared with a rate of only 2.2 haematomas per 1000 vaginal births in a cohort of 26 187 spontaneous or operative vaginal deliveries [368].

In women with VWD, vaginal bleeding is frequently reported to occur more than 2–3 weeks postpartum. The duration of bleeding after delivery in a normal patient is a median of 21–27 days [369–

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Table 18. Pregnancies in women with VWD.

Study	Population	Miscarriage	Prophylaxis	Postpartum haemorrhage	Perineal haematoma
Burlingame et al. [361]	5 pregnancies in 2 women	None	FVIII/VWF concentrates for 1/5	1/5 (20%)	None
Lak et al. [87]	100 women with type 3 VWD and had delivered at least 1 child	Rate not 'higher than... the general Iranian population'	FFP, cryo, FVIII/VWF concentrates	15/100 (15%)	Not reported
Caliezi et al. [362]	2 pregnancies in 1 woman with type 3 VWD	None	FVIII/VWF concentrates for 2/2	1/2 (50%) on day 15 from episiotomy	None
Kadir et al. [345]	84 pregnancies in 31 women	18 of 72 pregnancies not terminated	10/54 with 'no bleeding complications'	10/54 (18%) primary*; 6/54 (11%) required transfusion; 11/54 (20%) secondary†	3/54
Foster [339]	69 pregnancies in 31 women with VWD unresponsive to DDAVP	15 of 68 pregnancies not terminated	25/55 (46%) of those for whom data were available; FVIII/VWF concentrates (9); cryo (8); FFP (1)	In women who had type 2A, 2B or 3 VWD, 6/18 (33%) who were treated; 3/4 (75%) who were not treated	Not reported
Ramsahoye et al. [376]	24 pregnancies in 13 women	None reported (1 foetal demise at 38 weeks)	5 caesarean deliveries: cryo for 2/5; FVIII/VWF concentrate (Haemate-P‡) for 2/5; DDAVP for 1/5; 19 vaginal deliveries: cryo for 3/19, FVIII/VWF concentrate (Haemate-P‡; NHS 8Y) for 2/19	3/24 (12.5%) primary*; 6/24 (25%) secondary†; 2/6 secondary† had been treated	None
Greer et al. [329]	14 deliveries in 7 women with VWD	Not reported	Cryo (9)	5/9 who were treated (1 primary*, 3 secondary†, 1 both); 2/5 who were not treated (2 primary*)	1/14
Chediak et al. [363]	10 pregnancies in 6 women with VWD	3/10 pregnancies	Cryo for 5/7 deliveries; DDAVP for 2/7 deliveries	4/5 'massive'	1 had 'lumbar haematomas'
Conti et al. [390]	5 deliveries in 5 women with VWD	None	None	2/5 'late'	None

Cryo, cryoprecipitate; DDAVP, desmopressin; FFP, fresh-frozen plasma; FVIII/VWF, factor VIII/von Willebrand factor concentrate; NHS 8Y, National Health Service (UK) FVIII/VWF concentrate (8Y); VWD, von Willebrand disease.
*Primary, postpartum haemorrhage within the first 24 h after delivery.
†Secondary, postpartum haemorrhage after 24 h after delivery.
‡Haemate-P is the European equivalent of Humate-P.

371]. However, the VWF levels that are elevated during pregnancy return to baseline within 7–21 days [372,373], predisposing women with VWD to delayed postpartum haemorrhage. In the absence of a bleeding disorder, delayed or secondary postpartum haemorrhage is rare and occurs after <1% of deliveries [374,375]. In contrast, 20–25% of women with VWD had delayed postpartum haemorrhage, making delayed postpartum haemorrhage 15–20 times more common among these women than in normal subjects [345,376].

Among the published series of cases of women who were pregnant and had VWD (Table 18), multiple cases of postpartum haemorrhage occurred despite prophylaxis. The mean ± SD time of presentation of postpartum haemorrhage in women with VWD was estimated to be 15.7 ± 5.2 days after delivery [377]. The implication is that women with VWD may require frequent evaluation — and possibly prophylaxis — for 2 weeks or more postpartum. Weekly contact with these women is recommended during the postpartum period [347].

Management recommendations

The recommendations are graded according to criteria described in Table 1. Management Recommendations IV–X summarize the Panel's patient management recommendations. Evidence Tables 6–13 are provided in the full online document [10] for recommendations given a grade of B and having two or more references.

Management Recommendations IV

IV. Testing Before Treatment

A.   Before treatment, all persons suspected of having von Willebrand disease (VWD) should have a laboratory-confirmed diagnosis of type and severity of VWD. This recommendation does not preclude treatment that may be indicated for urgent or emergency situations, despite the absence of confirmatory laboratory data.
Grade C, level IV [87,133–136,148,394,395]

B.   Persons who do not have a definite diagnosis of VWD but who have von Willebrand factor ristocetin cofactor activity (VWF:RCo) levels of 30–50 IU dL^{-1} and have a bleeding phenotype may merit treatment or prophylaxis of bleeding in certain clinical situations.
Grade B, level III [191]

C.   Persons with activity levels of VWF:RCo higher than 10 IU dL^{-1} and factor VIII (FVIII) higher than 20 IU dL^{-1} should undergo a trial of desmopressin while in a nonbleeding state. Persons with levels below these thresholds are less likely to demonstrate clinical or laboratory responses to desmopressin, but a desmopressin trial should still be considered in these individuals.
Grade B, level IIa [101,218,220,221,224]

See Evidence Table 6 [10] for additional information and detail.

Management Recommendations V

V. General Management

A.   Treatment of persons who have von Willebrand disease (VWD) is aimed at cessation of bleeding or prophylaxis for surgical procedures.
Grade C, level IV [1,7,9]

B.   Continued bleeding, despite adequately replaced von Willebrand factor ristocetin cofactor (VWF:RCo) and factor VIII (FVIII) activity levels, requires evaluation of the person for other bleeding aetiologies, including anatomic.
Grade C, level IV

C.   Long-term prophylaxis is currently under investigation in an international cooperative study, and the long-term risks and benefits should be considered carefully.
Grade C, level IV [208,209]

D.   Individuals who are more than 2 years old, have VWD, and have not already been vaccinated should be immunized against hepatitis A and B.
Grade C, level IV [295]

E.   Persons with VWD should have the opportunity to talk to a knowledgeable genetic counselor.
Grade C, level IV [346]

F.   At diagnosis, persons with VWD should be counseled to avoid aspirin, other nonsteroidal anti-inflammatory drugs (NSAIDs), and other platelet-inhibiting drugs.
Grade C, level IV [296–298]

G.   Restriction of fluids to maintenance levels should be considered in persons receiving desmopressin (especially for young children and in surgical settings) to avoid the occurrence of hyponatraemia and seizures.
Grade C, level IV [258–260]

Management Recommendations VI

VI. Treatment of Minor Bleeding and Prophylaxis for Minor Surgery

A. Epistaxis and oropharyngeal, soft tissue, or minor bleeding should be treated with intravenous or nasal desmopressin, if appropriate, based on trial testing.
Grade B, level IIa [220,223,227,229,238]
  See Evidence Table 7 [10] for additional information and detail.

B. If elevation of von Willebrand factor (VWF) is necessary and response to desmopressin is inadequate, VWF concentrate should be used, with dosing primarily based on von Willebrand factor ristocetin cofactor activity (VWF:RCo) units and secondarily on factor VIII (FVIII) units.
Grade C, level IV [239,250]

C. For prophylaxis for minor surgery, initial treatment should be expected to achieve VWF:RCo and FVIII activity levels of at least 30 IU dL^{-1} and preferably higher than 50 IU dL^{-1}.
Grade B, level III [220,223,237,239]
  See Evidence Table 8 [10] for additional information and detail.

D. For minor surgery, VWF:RCo and FVIII activity levels of at least 30 IU dL^{-1} and preferably higher than 50 IU dL^{-1} should be maintained for 1–5 days.
Grade B, level III [239,245,246,253]
  See Evidence Table 9 [10] for additional information and detail.

E. For persons with VWD, management of minor bleeding (e.g. epistaxis, simple dental extraction or menorrhagia) with desmopressin and proper fluid restriction can be performed without laboratory monitoring unless desmopressin is used more than three times within 72 h.
Grade C, level IV [257,316]

F. For persons with mild to moderate von Willebrand disease (VWD), antifibrinolytics combined with desmopressin are generally effective for oral surgery. VWF concentrate should be available for persons who cannot receive desmopressin or who bleed excessively despite this combined therapy.
Grade B, level IIb [220,225,229,237–240]
  See Evidence Table 10 [10] for additional information and detail.

G. Topical agents, such as fibrin sealant or bovine thrombin, may be useful adjuncts for oral surgery in persons with VWD. Careful attention to haemostasis of an extraction socket and to suturing of sockets is also important in oral surgery in persons with VWD.
Grade C, level IV [237,293]

Management Recommendations VII

VII. Treatment of Major Bleeding and Prophylaxis for Major Surgery

A. All treatment plans should be based on objective laboratory determination of response of von Willebrand factor ristocetin cofactor (VWF:RCo) and factor VIII (FVIII) activity levels to desmopressin or to von Willebrand factor (VWF) concentrate infusion.
Grade B, level IIb [133,221,239,242–244,246–253,288,403]
  See Evidence Tables 11 and 12 [10] for additional information and detail.

B. Whenever possible, all major surgical procedures and bleeding events should be treated in hospitals with around-the-clock laboratory capability and with clinical monitoring by a team that includes a haematologist and a surgeon skilled in the management of bleeding disorders.
Grade C, level IV

C. For severe bleeding (e.g. intracranial, retroperitoneal) or for prophylaxis during major surgery, initial target VWF:RCo and FVIII activity levels should be at least 100 IU dL^{-1}. Subsequent dosing should maintain VWF:RCo and FVIII levels above a trough of 50 IU dL^{-1} for at least 7–10 days.
Grade B, level III [239,246,248–253,288,403]
  See Evidence Table 12 [10] for additional information and detail.

D. To decrease risk of perioperative thrombosis, VWF:RCo levels should not exceed 200 IU dL^{-1}, and FVIII activity should not exceed 250 IU dL^{-1}.
Grade C, level IV [277–279]

E. For major surgical procedures in selected patients with type 3 von Willebrand disease or acquired von Willebrand syndrome who are at risk for poor VWF recovery because of inhibitors, a preoperative trial infusion of VWF concentrate with pharmacokinetic laboratory monitoring should be considered.
Grade C, level IV

Management Recommendations VIII

VIII. Management of Menorrhagia and Haemorrhagic Ovarian Cysts in Women With VWD

A.   Women who have menorrhagia or abnormal vaginal bleeding should have a full gynaecologic evaluation before therapy.
Grade C, level IV [312]

B.   In an adolescent or adult woman who does not desire pregnancy but may desire future childbearing, the first choice of therapy for menorrhagia should be combined oral contraceptives.
Grade B, level III [339]

C.   In an adolescent or adult woman who does not desire pregnancy but may desire future childbearing, the first choice of therapy to prevent haemorrhagic ovarian cysts should be combined oral contraceptives.
Grade C, level IV [340,341,343]

D.   If a woman would otherwise be a suitable candidate for an intrauterine device, the second choice of therapy for menorrhagia should be the levonorgestrel intrauterine system.
Grade B, level IIb [404]

E.   For a woman who desires pregnancy, desmopressin, antifibrinolytics, or VWF concentrate may be tried to control menorrhagia.
Grade C, level IV [339]

F.   Dilation and curettage is not usually effective to manage excessive uterine bleeding in women with VWD.
Grade C, level IV [329,330]

Management Recommendations IX

IX. Management of Pregnancy and Childbirth in Women With VWD

A. Women planning for pregnancy should have, before conception, an evaluation with a haematologist and high-risk obstetrician, both of whom are skilled in the management of von Willebrand disease (VWD).
Grade C, level IV [346]

B.   Women with type 1, type 2, or type 3 VWD, with factor VIII (FVIII) or von Willebrand factor ristocetin cofactor (VWF:RCo) activity levels <50 IU dL^{-1} or a history of severe bleeding:

1.   Should be referred to a centre that has high-risk obstetrics capabilities and expertise in haemostasis for prenatal care, delivery, termination of pregnancy, or management of miscarriage.
Grade C, level IV

2.   Should receive prophylaxis with desmopressin or von Willebrand factor (VWF) concentrate before invasive procedures.
Grade C, level IV [346,347]

3.   Should achieve VWF:RCo and FVIII levels of at least 50 IU dL^{-1} before delivery and maintain those levels for at least 3–5 days afterward.
Grade C, level IV [9,212,339,345,347]

C.   If VWF:RCo and FVIII levels can be monitored and maintained above 50 IU dL^{-1} during labor and delivery, and no other coagulation defects are present, then regional anaesthesia may be considered.
Grade C, level IV [345]

D.   Because coagulation factors return to prepregnancy levels within 14–21 days after delivery, healthcare providers should be in close contact with women during the postpartum period.
Grade C, level IV [347]

Management Recommendations X

X. Acquired von Willebrand Syndrome

A.   Individuals who have acquired von Willebrand syndrome (AVWS) and who require surgery should be considered for a pharmacokinetic trial of therapy with desmopressin and/or von Willebrand factor (VWF) concentrate, with monitoring of von Willebrand factor ristocetin cofactor (VWF:RCo) and factor VIII (FVIII), to evaluate for possible accelerated clearance of VWF.
Grade C, level IV [114,152]

B.   For persons who have AVWS and who bleed excessively despite therapy with desmopressin and VWF concentrate, treatment with high-dose intravenous immunoglobulin (IGIV) should be considered, especially in IgG isotype monoclonal gammopathy of uncertain significance (MGUS). (See discussion of this use, not approved by the US Food and Drug Administration, in the section Treatment of AVWS.)
Grade B, level IIa [114,299–302]
   See Evidence Table 13 [10] for additional information and detail.

Opportunities and needs in VWD research, training and practice

Many recommendations in this guideline are based on relatively limited evidence, thus underscoring the need for further research. Some of these opportunities are discussed below.

Pathophysiology and classification of VWD

Determinants of VWF level and bleeding risk The risk of bleeding in persons with VWD depends on the level of functional VWF and on many other factors that are poorly understood. The plasma level of VWF can be influenced by mutations within or near the VWF gene. In addition, VWF levels depend on ABO blood type [45], possibly on the *Secretor* locus [111], and on hormonal status and stress, as discussed in The VWF Protein and its Functions *In Vivo*. Relatively few of the genetic and non-genetic determinants of VWF level have been characterized, and how they interact is not known. In addition, little quantitative information is available on the risk of specific bleeding symptoms as a function of the level of VWF in plasma. This information would be particularly useful for the management of patients who have VWF levels in the range of 30–50 IU dL^{-1}, for whom the risk of medically significant bleeding is not well defined.

VWF level in plasma alone does not account for the observed variation in bleeding symptoms, and recent studies are starting to uncover some of the underlying reasons. For example, persons who have both low VWF and defects in platelet aggregation have more severe bleeding [378]. Increased bleeding has also been associated with specific DNA markers for platelet membrane proteins [379]. It is likely that multiple haemostatic risk factors interact with VWF level in plasma to determine the likelihood of bleeding or thrombosis. Understanding these interactions and incorporating them into clinical practice will require additional basic, clinical, and epidemiological research.

Heterogeneity of type 1 VWD Partial quantitative deficiency of VWF can be caused by several mechanisms, as discussed in the section Classification of VWD Subtypes. Some persons have dominant VWF mutations that either decrease the secretion of VWF multimers or accelerate their clearance from the circulation. The prevalence of increased clearance as a cause of type 1 VWD is not known. Whether these different disease mechanisms correlate with distinct clinical features, including response to specific treatments, also is not known. Because type 1 VWD is the most common form of VWD, answers to these questions may have important consequences for medical practice.

Heterogeneity of type 2 VWD The concentration of haemostatically effective large VWF multimers can be selectively decreased by accelerated proteolysis or by various defects in multimer assembly [51]. These variants now are grouped together as type 2A VWD, but further subdivision of this category would be justifiable if specific mechanisms of disease were associated with different clinical symptoms or responses to therapy.

Most persons with type 2M VWD have been identified by finding a profound defect in ristocetin-induced binding to platelets associated with a normal VWF multimer pattern [57,69,74]. Defects in binding to collagen or other connective tissue elements could cause a similar bleeding phenotype, but the VWF:RCo assay is insensitive to such defects [75]. Collagen-binding abnormalities can be detected by the VWF:CB assays, but those assays are not used widely in the United States. The prevalence and medical importance of collagen-binding defects in type 2M VWD deserve further study.

Diagnosis and evaluation

Assessment of bleeding signs and symptoms The initial evaluation of patients for a medically important bleeding disorder can be difficult because mild bleeding is common in the healthy population. Specific symptoms have been assessed for clinical relevance in retrospective studies, and some appear to discriminate between healthy controls and persons with diagnosed bleeding disorders (Table 9 and Fig. 3). However, the utility of these questions must be established prospectively for less highly selected persons.

Quality and availability of laboratory testing Reliable testing for VWF:Ag and FVIII is widely available, but VWF:RCo, RIPA and VWF multimer analysis are much more variable in their performance characteristics and can be difficult to obtain. Also, tests of VWF:FVIIIB are offered by few laboratories. More robust methods for assessing VWF function and multimer structure must be developed for routine use in the diagnosis of VWD. In addition, the sensitivity and specificity of test ratios such as VWF:RCo/VWF:Ag should be established for identifying the qualitative defects that characterize type 2A and type 2M VWD. Criteria should be established for VWF

multimer analysis to distinguish a significant decrease in large multimers (in types 2A and 2B VWD) from a substantially normal multimer distribution (in types 1, 2M and 2N VWD).

VWF gene sequencing Mutations that cause many types of VWD can be identified by sequencing the VWF gene in DNA samples from patients [24]. The locations of mutations appear to correlate well with some disease phenotypes, suggesting that DNA sequencing could be a useful diagnostic method in VWD. With appropriate study and experience, DNA sequencing may become economical and feasible for routine use. In addition, the widespread application of VWF gene sequencing would provide invaluable information about the prevalence of VWF mutations as a function of VWF level, the strength of the relationship between VWF genotype and VWD phenotype, the penetrance of specific mutations, and the biochemical mechanisms that cause VWD. This knowledge would also be an outstanding resource for the identification and characterization of other factors that modify bleeding symptoms in VWD.

Management of VWD

Many of the standard treatments for VWD have limited experimental support. For example, the intensity and duration of therapy necessary to control bleeding have not been established for many clinical situations and often have been extrapolated from anecdotal experience in haemophilia. The indications for prophylaxis of bleeding are not well defined. These issues should be addressed by appropriate clinical studies.

Desmopressin Many persons with VWD respond to desmopressin with a clinically useful rise in VWF and FVIII, but the likelihood of a good outcome depends on the type of VWD and the underlying biochemical mechanism of disease. In type 1 VWD, persons who have accelerated clearance of plasma VWF may have a transient response to desmopressin [43,225]. Whether desmopressin should be used at all in persons with type 2B VWD is controversial [226,234,254,255,380–383]. In type 2N VWD, the baseline FVIII level may be a good predictor of the magnitude and duration of the FVIII response to desmopressin [79,224,228,384]. The drug is thought to be safe for use in pregnancy, but the published experience in this setting is limited [268,269]. Hyponatraemia and thrombotic events have occurred after desmopressin, but risk factors for these events and their incidence have not been established. These important clinical issues should be addressed by studies of desmopressin in specific types of VWD. In addition, the availability of desmopressin for s.c. administration may improve management of VWD.

Factor concentrates The available plasma-derived products that contain VWF also contain FVIII as part of the FVIII–VWF complex, and only two such products are currently licensed in the United States for treatment of VWD. When administered to patients with VWD, the infused FVIII may add to the endogenous FVIII production and cause markedly elevated FVIII levels that are much greater than the VWF levels achieved with treatment; these have been associated with thrombosis [212]. High FVIII levels can be avoided by adjusting the dose of product administered, but VWF levels then may be relatively low. Whether FVIII or VWF levels or both, should be used to monitor treatment with FVIII–VWF concentrates is unknown. Use of a pure VWF product in place of FVIII–VWF concentrates would avoid the disproportionate increase in FVIII. A pure VWF concentrate has been used in Europe [385] but is not currently available in the United States. Studies are needed to establish appropriate treatment and monitoring regimens for these products. In addition, prelicensure studies of recombinant VWF are needed to establish its safety, efficacy and role in the treatment of VWD. The licensing of other products containing both VWF and FVIII would also enhance therapeutic options.

Platelets Approximately 15% of the total VWF in blood is found within platelets, and platelet VWF appears to contribute to haemostasis. Although VWD patients who have abnormal or low platelet VWF have been described, there has been only limited exploration of the feasibility and utility of such testing, in part because of limitations of practical methods. Clinically, platelet transfusions have been reported to stop bleeding in some patients with VWD who were not helped by transfusion of FVIII-VWF concentrates [289,290]. The efficacy and appropriate use of platelet transfusions in persons with VWD or AVWS need to be established.

Antifibrinolytics Tranexamic acid and aminocaproic acid have been used alone or as adjunctive therapy to treat bleeding in VWD. The safety, efficacy, and optimal dosing of these agents in VWD should be established by suitable clinical studies. In addition, the availability of orally administered tranexamic acid would broaden the therapeutic options for antifibrinolytic therapy.

Gene therapy of VWD

Severe type 3 VWD potentially can be treated with gene therapy. The gene for VWF is larger than could easily be introduced into many vectors, but gutless adenoviral vectors could easily accommodate a gene the size of VWF (8.5 kb). The prevalence of type 3 VWD and its clinical symptoms, however, does not place it in a high-priority category for gene therapy trials. Point mutation repair initially was an exciting approach for VWD [386,387], but follow-up studies have not achieved the same rate of success *in vitro* [388,389].

Issues specific to women

VWF is particularly important for haemostasis during menses and at childbirth. Consequently, women are affected disproportionately by having VWD, especially during their child-bearing years.

Menorrhagia The incidence of menorrhagia appears to vary inversely with VWF level, independent of whether women meet criteria for having VWD [47]. Because menorrhagia is so common, even a small reduction in its severity could have important implications for women's health. As discussed in the section Menorrhagia, several treatments have been used for menorrhagia associated with VWD, but their efficacy has not been demonstrated convincingly. Therefore, clinical studies would be useful to establish the effect of VWF level on menorrhagia and to evaluate specific treatments for women who have VWD or low plasma levels of VWF.

Labour and delivery Several small case series indicate that women who have VWD and VWF levels <50 IU dL^{-1} at delivery have an increased incidence of immediate and delayed postpartum haemorrhage. These complications appear to be prevented by replacement therapy with FVIII–VWF concentrate before delivery and by either concentrate or desmopressin in the postpartum period [87,345,390]. How the risk of bleeding correlates with VWF level or FVIII level is not known, and the required intensity and duration of therapy have not been established.

Training of specialists in haemostasis

In the United States, despite scientific progress in basic and clinical research in bleeding and thrombotic disorders, including VWD, there is a shortage of skilled clinicians and laboratorians with expertise in haemostasis [391]. Training opportunities need to be developed and expanded for haemostasis specialists. Recent clinical training opportunities include a new NHLBI initiative for training in non-malignant haematology [392] and a recent clinical fellowship initiative from the National Hemophilia Foundation [393]. Recognition of haemostasis as a bona fide clinical and laboratory subspecialty in the United States could enhance entry into the field.

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