CLINICAL INVESTIGATION

New developments in the diagnosis and treatment of von Willebrand disease

von Willebrand disease (VWD) arises from deficiencies and/or defects in the plasma protein VWF. VWD is reportedly the most common inherited bleeding disorder. The classification scheme for VWD identifies six types, with type 1 VWD defining quantitative deficiencies of VWF, type 3 defining an absence of plasma VWF, and types 2A, 2B, 2M and 2N defining qualitative defects of VWF. Diagnosis requires a clinical evaluation, with evidence of both familial and personal history of (primarily mucocutaneous) bleeding, as well as laboratory assessment using a wide panel of tests, including several activity-based assays. Management of VWD typically entails standard therapy, employing desmopressin wherever possible, factor concentrates in other situations, and antifibrinolytic and additional therapies as required. Some management strategies are modified according to geographic locality. There are several developments in both the diagnosis and management of VWD that may influence the future landscape.

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von Willebrand disease & VWF

von Willebrand disease (VWD) arises from deficiencies and/or defects in the plasma protein VWF [1–4]. VWD is believed to represent the most common inherited bleeding disorder and is classified into six different types. These are: type 1 (which represents a partial quantitative deficiency of VWF); type 3 (which defines a 'total' deficiency of VWF), and types 2A, 2B, 2M and 2N (with each characterizing VWD-type distinctive qualitative defects). The classification is primarily based on phenotypic assays: mainly the FVIII, VWF antigen (VWF:Ag) and various VWF activity assays, as supplemented by additional testing on a case-by-case or locality basis with the VWF multimer, ristocetin-induced platelet agglutination (RIPA) and VWF:FVIII binding (VWF:FVIIIB) assay (Tables 1–3) [1–7]. VWF activity is usually assessed by ristocetin cofactor (VWF:RCo) or collagen binding (VWF:CB) assays. In select cases, genetic analysis may also be undertaken. A variety of approaches and additional assays may also be utilized, depending on the level of clinical suspicion, the laboratory experience or preference, and the geographic locality. Diagnosis of congenital VWD in an individual also requires the establishment of a personal and familial history of bleeding, although bleeding tendency in individuals with presumptive VWD may vary for reasons other than level and activity of VWF [7]. Therefore, it is also important to recognize and consider the contribution of potential compound defects (including platelet-related disorders or reduced platelet receptor density). VWD and platelet function defects in particular may also require application of a differential diagnostic process given the broad similarities in bleeding symptoms

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Table 1. Summary of current tests (and future directions) in von Willebrand disease diagnosis.			
Test	What it measures	Comments	The future
FVIII:C	Coagulant (functional) activity of FVIII	Standard laboratory test. Usually performed by one-stage clotting assay. Usually low in VWD, but may be normal. Reduction in FVIII:C tends to parallel reductions in VWF	Some movement towards performing FVIII chromogenic tests, but FVIII:C testing likely to remain largely as is
VWF:Ag	VWF protein level	Quantitative, but nonfunctional assessment of VWF. Typically performed by ELISA or LIA technology. Low in type 1 VWD, usually low (but may be normal) in type 2 VWD, absent in type 3 VWD	Will remain a core laboratory test in VWD diagnosis, but there will be continued movement away from ELISA towards LIA technology
VWF:CB	level of VWF:CB	Quantitative functional assay. Measures adhesion of VWF to collagen. Typically performed by ELISA technology. Low in type 1 VWD and parallels VWF:Ag, usually low in type 2 VWD (depends on subtype – always low in 2A VWD, and much lower than VWF:Ag), absent in type 3 VWD	Will gain increased acceptance in VWD diagnosis. May become adapted to LIA or other technology
VWF:RCo	VWF:RCo level	Quantitative functional assay. Measures adhesion of VWF to platelets using ristocetin as facilitator. Typically performed by agglutination technology, either with an aggregometer or an automated analyzer. Low in type 1 VWD and parallels VWF:Ag, usually low in type 2 VWD (depends on subtype – always low in 2A VWD, and lower than VWF:Ag), absent in type 3 VWD	Inappropriate movement away from this assay because of test limitations (i.e., high complexity, variability and time consuming), in some cases inappropriately replacing with VWF:Act. Attempts to develop as ELISA format failed to capture general interest. Future improved assays being developed without ristocetin for use on automated analyzers
VWF multimers	VWF multimer distribution and structure	Qualitative assessment of VWF structure. Loss of HMW VWF is characteristic of types 2A and 2B VWD. Structural abnormalities may also be present in some type 2 VWD cases	High complexity and time-consuming nature leading to high error rates related to interpretation and ongoing attrition of usage in general VWD diagnostics
VWF:Act	VWF binding to a monoclonal antibody	Name has been designated by the manufacturer, with claims that the assay reflects VWF activity; however, workers in the field dispute this claim	Will retain some popularity in short term due to ease of use and favorable technical considerations; may be replaced by emerging VWF:RCo-like assays in the future
VWF:pp	VWF:pp level	Quantitative assessment of VWF propeptide. Use of this test, often as a ratio to VWF:Ag, may have utility in assessment of VWF disorders related to clearance mechanisms	Will likely gain more widespread acceptance over time, but continued take-up awaits further evidence of clinical utility as well as improved (automatable) technology
PFA-100®	Platelet adhesion and aggregation in whole blood	Global assay that has some sensitivity to VWD. Also potentially useful in context of desmopressin challenge	Possible expansion of test cartridges to increase test utility. Remodeled as a PFA-200. Increasing usage in context of desmopressin trials are probable

FVIII:C: FVIII coagulant; HMW: High-molecular weight; LIA: Latex immunoassay; VWD: von Willebrand disease; VWF:Ag: VWF antigen; VWF:CB: VWF collagen binding; VWF:pp: VWF propeptide; VWF:RCo: VWF ristocetin cofactor.

[8].

VWF has two primary hemostatic functions; these are:

- To promote adhesion of platelets to one another and also to the vasculature (this defines 'primary' hemostasis);
- To bind, stabilize and protect FVIII (which is then delivered by VWF to the site of need and then used to facilitate 'secondary' hemostasis).

Nevertheless, VWF performs several additional functions to facilitate arrest of bleeding following injury, and thus contains many functional binding sites in addition to those classically recognized as facilitating binding to its major platelet receptor glycoprotein Ibα (GPIBA) and to FVIII. These

Table 2. Phenotypic problems associated with diagnosis of von Willebrand disease.		
Problem	**Consequence**	**Strategies**
Continuum of VWF values in all normal individuals, as well as most individuals with VWD	Leads to overlaps in values between unaffected 'normals' and affected 'VWD' individuals (i.e., potential false-positives and -negatives for VWD)	Be aware of these issues and recognize that a low level of VWF does not necessarily 'diagnose' VWD, and that a normal level of VWF does not necessarily 'exclude' VWD. Repeat all tests at least once for confirmation of initial findings
Limitations of typical methods of establishing test reference ranges	By definition, these will ascribe ~2.5% of the normal population as having a low level of VWF (i.e., potential false-positives for VWD)	
VWF is an acute-phase reactant, and increases in times of stress, illness, infection, as well as during pregnancy	May mask a true VWF deficiency based on testing during an inappropriate time point (i.e., potential false-negatives for VWD)	
Many of the assays used to identify VWD, particularly VWF:RCo, show a high degree of assay variability	Testing will generate differing test results on different occasions in the same patient tested at different times or in different laboratories. For example, discordance by RCo/Ag will occasionally not be observed in types 2A, 2M and 2B VWD (leading to misdiagnosis as type 1), or a false functional discordance may instead be identified (leading to misdiagnosis of type 1 as 2A)	Be aware of individual test limitations. Use extensive test panels. The addition of VWF:CB to a core test panel of VWF:RCo, VWF:Ag and FVIII:C, for example, will reduce phenotypic-based diagnostic error rates by approximately half. Repeat laboratory tests on a fresh sample for confirmation. Use data from a desmopressin challenge to confirm a diagnosis or VWD-type assignment
LOD issues are evident for many VWF assays. Particularly VWF:RCo, which may be as high as 20 U/dl, or VWF:Ag when measured by certain technologies (e.g., LIA)	Can cause misidentification of (the most severe forms of) VWD, and poor discrimination of type 3, severe type 1 and severe type 2 VWD, either because they cannot be differentiated, or because of falsely identified concordance/ discordance (e.g., using RCo/Ag)	
Most laboratories use inappropriate or limited test panels	These panels are insufficient to properly identify phenotypically all cases of VWD, and will on occasion lead to VWD misdiagnosis	
Functional assays for VWF do not all measure the same property of VWF, nor do they have the same utility to identify qualitative VWD disorders. Some laboratories inappropriately use some assays as surrogates for others (e.g., use of monoclonal antibody-based assays as a surrogate for VWF:RCo)	Some laboratories may misdiagnose VWD based on poor choice of assay or test panel	
There are differences in terms of commercial offerings purporting to measure the same functional activity (e.g., not all assays commercially marketed as VWF:CB assays have the same ability to discriminate type 1 and 2 VWD)		
Preanalytical issues in VWD testing	These may lead to identification of VWD-like patterns in normal individuals, or to incorrect VWD types being assigned to VWD individuals. Usually causes normal individuals or type 1 VWD individuals to be misidentified as type 2	Follow expert guidelines and use stringent collection and sample processing protocols

FVIII:C: FVIII coagulant; LIA: Latex immunoassay; RCo/Ag: VWF ristocetin cofactor to antigen ratio; VWD: von Willebrand disease; VWF:Ag: VWF antigen; VWF:CB: VWF collagen binding; VWF:RCo: VWF ristocetin cofactor.

include binding sites for other platelet receptors (e.g., αIIbβ3) and subendothelial matrix components such as collagen. Thus, defects in these regions of the VWF molecule can also lead to VWD, and may require additional test strategies. The steady-state plasma concentration and structural composition of VWF is

Table 3. Classification scheme for von Willebrand disease: laboratory diagnosis, diagnostic challenges and desmopressin response profiles.

VWD type	Description	Comments	Laboratory diagnosis	Diagnostic challenges	Desmopressin response profile
1	Partial quantitative deficiency of VWF	Most common presentation of 'VWD' to most laboratories, with most patients presenting with mildly reduced levels of VWF	Low levels of VWF, with VWF functional concordance (i.e., ratio of functional VWF/VWF:Ag approximates unity). RIPA usually normal unless VWF <20 U/dl	Sometimes misidentified as type 2 VWD (e.g., due to LOD issues; assay variability, especially VWF:RCo; or preanalytical issues) or misidentified as not VWD (e.g., due to acute-phase increase in VWF due to stress or pregnancy)	Usually respond well, unless VWF <10 U/dl. Generally, VWF:Ag, VWF:RCo, VWF:CB and FVIII all appreciably rise (2–4x baseline), with CB/Ag and RCo/Ag ratios remaining >0.7
2A	Decreased VWF-dependent platelet adhesion and a selective deficiency of HMW VWF multimers	Globally considered to be the most common presentation of type 2 VWD	Loss of HMW VWF. Usually low levels of VWF, with VWF functional discordance (i.e., ratios of RCo/Ag and CB/Ag are typically <0.7)	Sometimes misidentified as type 1 or 2M VWD (e.g., due to LOD issues; assay variability, especially VWF:RCo; or limited test panels)	Variable clinical response (some efficacy in some patients). Typically, VWF:Ag and FVIII rise (2–4x baseline), but VWF:RCo and VWF:CB do not, with CB/Ag and RCo/Ag ratios remaining <0.7
2B	Increased affinity of VWF for platelet glycoprotein Ib	Rare form (generally 10–20% of type 2 VWD (~1–5 cases per million population). Defined by enhanced responsiveness in a RIPA assay	Low to normal levels of VWF, typically with VWF functional discordance (i.e., ratios of RCo/Ag and CB/Ag generally <0.7), loss of HMW VWF and (mild) thrombocytopenia. Atypical cases may not show this pattern	Sometimes misidentified as type 2A VWD (especially if RIPA not performed). Sometimes misidentified as type 1 VWD (especially if RIPA or VWF:CB not performed)	Use is contentious – believed by some to be contraindicated, whereas others believe it represents an effective treatment in a proportion of patients. Effect of desmopressin on VWF and FVIII depends on defect. All parameters will rise initially, but may fall quickly depending on clearance mechanisms and defect
2M	Decreased VWF-dependent platelet adhesion without a selective deficiency of HMW VWF multimers	Under-recognized form of type 2 VWD. Globally considered a relatively uncommon form of type 2 VWD, but some workers believe 2M VWD is at least as common as 2A VWD	Low to normal levels of VWF, usually with VWF functional discordance detected by RCo/Ag being <0.7, but CB/Ag being relatively normal. HMW VWF are present, but multimers may show other abnormalities	Can be misidentified as type 1 or 2A VWD (e.g., due to LOD issues; assay variability, especially VWF:RCo; limited test panels that omit VWF:CB or multimers)	Variable clinical response (some efficacy in some patients). Typically, for platelet-binding defect cases, VWF:Ag, VWF:CB and FVIII all rise (2–4x baseline), but VWF:RCo does not, with CB/Ag ratio remaining >0.7 and RCo/Ag ratio remaining <0.7

CB/Ag: VWF collagen binding to antigen ratio; HMW: High-molecular weight; RCo/Ag: VWF ristocetin cofactor to antigen ratio; RIPA: Ristocetin-induced platelet agglutination; VWD: von Willebrand disease; VWF:Ag: VWF antigen; VWF:CB: VWF collagen binding; VWF:FVIIIB: VWF:FVIII binding; VWF:RCo: VWF ristocetin cofactor.
Classification scheme derived and adapted from [1]. Table modified with permission from [9].

Table 3. Classification scheme for von Willebrand disease, laboratory diagnosis, diagnostic challenges and desmopressin response profiles (cont.).					
VWD type	Description	Comments	Laboratory diagnosis	Diagnostic challenges	Desmopressin response profile
2N	Markedly decreased binding affinity for FVIII	Rare form (generally <10%) of type 2 VWD (~1–5 cases per million population)	Defined by VWF:FVIIIB assay, with low FVIIIB/VWF ratios	Sometimes misidentified as hemophilia A/hemophilia A carrier, or other forms of VWD (especially type 1)	Variable clinical response (some efficacy in some patients), depending on composite defects present
3	Virtually complete deficiency of VWF	Rare form of VWD in developed countries (~1–5 cases per million population), but disproportionately more common in developing countries	Typically defined by VWF levels <2 U/dl and FVIII <10 U/dl	Sometimes misidentified as (severe) type 1 VWD (e.g., LOD issues) or hemophilia A (if VWF testing not performed)	Ineffective

CB/Ag: VWF collagen binding to antigen ratio; HMW: High-molecular weight; RCo/Ag: VWF ristocetin cofactor to antigen ratio; RIPA: Ristocetin-induced platelet agglutination; VWD: von Willebrand disease; VWF:Ag: VWF antigen; VWF:CB: VWF collagen binding; VWF:FVIIIB: VWF:FVIII binding; VWF:RCo: VWF ristocetin cofactor. Classification scheme derived and adapted from [1]. Table modified with permission from [9].

controlled by a complex process that involves manufacture, storage, secretion, proteolysis and clearance of VWF [1]. Only some of the mechanisms involved have been elucidated, although ADAMTS13 plays a key role. The heterogeneity of VWD can, therefore, be considered to be reflective of the large and complex multimeric protein that VWF represents. Moreover, many factors in addition to the *VWF* gene can influence a individual's phenotypic presentation and their bleeding risk or clinical presentation [7]. All of these considerations in total mandate a comprehensive test strategy to appropriately facilitate the diagnosis or exclusion of VWD.

Phenotypic characterization of VWD: the theory
Type 1 VWD is defined as a partial deficiency of VWF, but showing similar (or 'concordant') reductions in most test parameters; notably, VWF:Ag, FVIII coagulant [FVIII:C], VWF:RCo and VWF:CB (Tables 1–3). Multimer analysis shows a normal multimeric structure and distribution of VWF. RIPA would typically be normal unless levels of VWF fall below approximately 10–20 U/dl.

Type 3 VWD is defined as an absence of plasma VWF. Classically this means a total absence of VWF, but given testing realities and VWD heterogeneity, a 'virtually' total absence may also be acceptable for its diagnosis. Thus, all VWF tests (i.e., VWF:Ag, VWF:RCo and VWF:CB assays) would yield values approximately 0 U/dl (or <5 U/dl in practice). Multimer analysis would show a lack of VWF, and RIPA would be absent (i.e., there would be no response to ristocetin).

Type 2 VWD is defined by qualitative VWF abnormalities. VWF:Ag is usually low, but it may be normal (depending on the type of VWD). However, VWF activity-based tests will show an abnormality or reduction. Importantly, the overall test patterns will help to identify the 'specific' VWD type [1–5,9,10]. This can be summarized as follows:

■ In type 2A VWD, the defect is caused by a loss of high-molecular weight (HMW) VWF, due either to faulty assembly or enhanced proteolysis [1]. The deficiency of HMW VWF can be identified using multimer analysis, or by assessing selective VWF activity assays, with values of VWF:RCo and VWF:CB both lower than VWF:Ag [11]. This test pattern is termed VWF functional discordance, and is expressed numerically using assay ratios. Type 2A VWD is, therefore, typically characterized by VWF:RCo/VWF:Ag (RCo/Ag) and VWF:CB/VWF:Ag (CB/Ag) ratios below approximately 0.6–0.7 [1–5,9–11].

- Type 2B VWD is instead characterized by enhanced binding (i.e., hyper-adhesive activity) of VWF to GPIBA. This is classically identified by laboratory testing as an elevated RIPA responsiveness, or a response to ristocetin at a low dose of reagent [1]. This enhanced VWF–GPIBA binding will often lead to *in vivo* clearance or loss of HMW VWF (the most adhesive forms) from the circulation. It will also lead to clearance or loss of the VWF-bound platelets (and thus to mild thrombocytopenia). However, some 'atypical' forms of 2B VWD exist and these may not show some or all of these latter features [12–14]. As per type 2A VWD, the loss of HMW VWF can be identified using multimer analysis, or alternatively using surrogate markers of HMW VWF such as VWF:CB and VWF:RCo [1–5,9–11]. Type 2B VWD cases, therefore, typically express RCo/Ag and CB/Ag ratios under approximately 0.6–0.7. This is particularly so under periods of stress, given that VWF is an acute-phase reactant, and this pattern may thus lead to some individuals with 2B VWD to be misidentified as 2A VWD should RIPA testing not be performed. Also, although an enhanced RIPA response characterizes 2B VWD, an enhanced RIPA response does not necessarily define type 2B VWD. Some cases of enhanced RIPA response will actually reflect platelet-type (PT)-VWD (see below) [15], and other cases will be false-positives and potentially reflect high levels of 'functionally normal' VWF [16].

- Type 2M VWD represents an inherent VWF defect where loss of VWF function is not due to loss of HMW VWF [1]. Thus, although multimer analysis may show some structural abnormalities, HMW multimers will be present, and the test pattern may more closely resemble type 1 VWD. Most cases of reported type 2M have defective binding to GPIBA; hence, VWF:RCo, which 'functionally' reflects this binding, tends to be lower than VWF:Ag, and RCo/Ag ratios are below 0.7 [1–5,17–19]. However, since VWF:CB is less affected, its values are similar to VWF:Ag (i.e., show concordance), and CB/Ag ratios are typically above 0.7. Thus, in summary, most cases of 2M VWD will be identified with low RCo/Ag but normal CB/Ag. Interestingly, rare (but increasingly reported) forms of 2M VWD show the opposite pattern, since the VWF defect affects VWF:CB but not GPIBA binding; phenotypically this leads to low CB/Ag ratios, but normal RCo/Ag ratios [1–5,20–24].

- Type 2N VWD represents an inherent VWF defect that causes defective binding to FVIII [1]. Accordingly, circulating (unbound) plasma FVIII is labile and prone to proteolysis; this causes plasma levels of FVIII:C to be lower than VWF, and this leads to low plasma FVIII/VWF ratios. 2N VWD tends to mimic hemophilia A (or hemophilia A carriers) by laboratory phenotype, and thus may be misdiagnosed as such by clinicians. 2N VWD can, however, be distinguished from hemophilia A (or hemophilia A carriers) by performance of the VWF:FVIIIB assay. Using this assay, low FVIIIB/VWF ratios will define 2N VWD, but normal ratios may alternatively help to define hemophilia A (or hemophilia A carriers) [25].

PT-VWD is a rare disorder that reflects a defect not of VWF but of platelet GPIBA. It is thus not considered a 'true' form of VWD, but rather a platelet-function disorder. Nevertheless, PT-VWD (which reflects a hyper-adhesive GPIBA) yields test patterns very similar to type 2B VWD (which reflects a hyperadhesive VWF). In summary, these patterns are: elevated RIPA responsiveness; loss of circulating HMW VWF and platelets; and low ratios of both RCo/Ag and CB/Ag. PT-VWD and 2B VWD can only be phenotypically distinguished by using a RIPA mixing assay, or by evaluating VWF binding using highly specialized studies [14,15,26,27]. Alternatively, PT-VWD and 2B VWD can be differentiated by molecular genetic testing of the *GPIBA* or *VWF* genes [6,7,28].

Phenotypic 'mischaracterization' of VWD: the practice

Although phenotypic characterization of VWD is critical to its appropriate diagnosis, phenotypic evaluations sometimes lead to ineffective diagnosis or to misdiagnosis of VWD because of a variety of issues as largely summarized in Tables 2 & 3. Considering these factors, and dependent on the tests and test panels utilized by laboratories and the resultant test results they obtain, several VWD misdiagnoses may ensue. Typically, patients with a mild form of type 1 VWD may easily be misidentified as not having VWD (false-negative); individuals that do not actually have VWD may be misdiagnosed as having VWD (false-positive); individuals with type 3 VWD may be misdiagnosed as having severe type 1 or 2 VWD or as hemophilia A; individuals with type 1 VWD may be misdiagnosed as having type 2 VWD; and individuals with type 2 VWD might be misdiagnosed as having type 1 VWD [29–36].

Unfortunately, performance of VWF multimers will not 'prevent' many of these misdiagnoses. In fact, a large proportion of laboratories performing multimer analysis identify 'false' loss of HMW VWF in normal or type 1 VWD samples (i.e., false type 2 VWD), or on other occasions will miss the loss of

HMW VWF in a type 2 VWD case [37–40]. Expert centers using multimer analysis are not immune to the potential misdiagnosis of VWD using multimer analysis, given that approximately 20% of cases identified with type 1 VWD in recent molecular analysis studies could alternatively be defined as being type 2 VWD (mostly as 2M VWD) [41]. This can be explained by false VWF functional concordance identified using VWF:RCo and VWF:Ag assays (and due to high assay variability and assay limit of VWF detection issues), and multimer analysis failing to resolve the diagnosis between types 1 and 2M VWD.

Other useful investigative approaches

Diagnostic problems arising from laboratory tesing can be minimized by several strategies, which can be summarized as: use of improved methodologies; extended test panels; repeated testing using fresh blood samples for confirmation; testing of nonstressed, ill or pregnant patients (which may lead to false high or normal levels of VWF); stringent collection and sample processing protocols; and, data from a desmopressin challenge (Table 2).

Desmopressin challenge test as an aid to diagnosis of VWD & assignment of VWD type

Desmopressin (1-desamino-8-D-arginine vasopressin) is a nontransfusional form of therapy given to select patients with VWD that results in the release of endogenous (endothelial-cell stored) VWF. Desmopressin is (nearly invariably) effective in type 1 VWD where VWF levels are above 30 IU/dl, raising levels above 50 U/dl for several hours. Desmopressin is sometimes effective in cases of type 1 with lower levels of VWF, and sometimes also in type 2 VWD [42]. Thus, a trial of desmopressin is commonly performed to assess clinical utility in these patients. Importantly, desmopressin responsiveness is typically stable over time within individuals, although it will vary between individuals. Assessing data from desmopressin trials has additional (but broadly under-recognized) utility as part of the diagnosis and typing strategy of VWD, given that the desmopressin response profile is often VWD-type characteristic [3,4,10,17–19]. In brief, in type 1 VWD, FVIII and all VWF test parameters will rise post-desmopressin, and RCo/Ag and CB/Ag ratios will be above 0.7 both prior to and post-desmopressin. In type 2A VWD, FVIII and VWF:Ag will rise post-desmopressin, but VWF:RCo and VWF:CB will not, or their response will be weak and/or transient; therefore, RCo/Ag and CB/Ag ratios typically below 0.7 pre-desmopressin will remain below 0.7 post-desmopressin. In GPIBA-binding dysfunction type 2M VWD, FVIII, VWF:Ag and VWF:CB, typically all

rise post-desmopressin; however, VWF:RCo does not, or will only weakly rise. Therefore, in type 2M VWD, CB/Ag ratios typically remain above 0.7 but RCo/Ag ratios remain below 0.7, both pre- and post-desmopressin.

In summary, desmopressin response patterns can help assign the VWD type in those individuals in which the VWD type is unclear. The PFA-100˚ (Siemens, Marburg, Germany) also has potential utility in this setting. This instrument is a platelet-function screening tool that is very sensitive to VWF activity and thus to VWD. With this instrument, initially prolonged closure times will tend to shorten and normalize post-desmopressin in type 1 VWD, but not in types 2A and 2M VWD [19]. There may also be value in assessing post-desmopressin test patterns relating to VWF:Ag and the VWF propeptide (VWF:pp) because of increased VWF clearance, for example, being observed in types 1 and 2B VWD [43].

Genetic testing in VWD

Genetic testing in VWD is not broadly recommended in VWD diagnosis, for reasons expanded elsewhere [6,7,28]. In brief, there are many additional influences to, or modifiers of, plasma VWF level and function, to the *VWF* gene. These include: the ABO blood group; platelet and endothelial cell activity; epigenetic events; hormonal influences (e.g., menstrual and pregnancy related); ADAMTS13 level and activity; known or yet to be identified factors related to the manufacture, storage, secretion, proteolysis and clearance of VWF; and a multitude of environmental factors that contribute to raise or reduce VWF levels, including stress, exercise, medications, illness, disease and inflammation. Thus, there is a large disconnection between the *VWF* genetic profile (i.e., mutations and polymorphisms) and the laboratory-measured phenotype. The likelihood of successful genetic testing is also low for most VWD investigations (comprising either mildly reduced VWF/type 1 VWD or non-VWD). Given the large gene size and VWD heterogeneity, genetic testing is also likely to require an expensive and exhaustive evaluation of the entire *VWF* gene. Finally, the clinical utility is also low in most investigations, given that the usual treatment choices are limited and similar (i.e., desmopressin and/or VWF concentrate).

In summary, since most cases of VWD will not yield a positive genetic finding, there is a high false-negative risk in genetic testing; however, genetic testing may be useful in a few select investigations [6,7,28,44], primarily type 2N (to help discrimination from hemophilia A or hemophilia A carriers), type 2B VWD (primarily for discrimination from PT-VWD),

type 3 VWD (for prenatal assessment/family studies and alloantibody risk assessment), and sometimes in type 2A/2M VWD or in type 1 VWD where VWF levels are <20–30 IU/ml. Genetic testing in these cases is focused and there may be therapeutic implications to an incorrect diagnosis (e.g., VWF concentrate vs FVIII concentrate in 2N VWD vs hemophilia A; VWF concentrate vs platelet replacement in 2B VWD vs PT-VWD).

Global perspectives in diagnosis & prevalence of VWD & VWD types

■ Global prevalence of VWD

VWD is globally recognized to be the most common inherited bleeding disorder [2], more common even than hemophilia A, which has a reported prevalence of approximately 100 per million male population [45]. However, given the difficulties in diagnosis of VWD, it is not surprising that different prevalence rates have been ascribed in different geographies [2]. Moreover, the perceived prevalence of VWD depends on the method used to capture data. High prevalence rates for VWD of up to 1% of the general population have been obtained using epidemiological/population-screening studies. However, very few people with 'lowish' levels of VWF, as potentially identified by laboratory testing, will have an identifiable bleeding disorder [31] and actively present themselves for clinical investigation; hence, prevalence data collected by the more usual approach (case referral or bleeding disorder registry data) would reflect much lower prevalence rates approaching approximately one in 10,000 individuals. Moreover, prevalence data, in part, further reflects the many other geographical or locality based considerations, such as tests and test panels employed, clinical awareness and economic considerations [2].

■ Frequency of VWD types

Similarly to VWD prevalence data, large variations in VWD-type data are reported, and based in part on the variations in tests and test panels used by individual laboratories, as well as geographical considerations such as culture (e.g., acceptance of consanguinity leading to increases in familial and type 3 VWD), definitions (e.g., inclusion or exclusion of cases of 'mild' or 'possible' type 1 VWD), and issues related to laboratory testing (e.g., limited test panels or investigation of only the most severe bleeding problems in developing countries).

In most reports from developed countries, type 1 VWD tends to predominate (typically ranging from ~40–90% of all VWD cases), whereas in developing countries, type 3 VWD cases (rare in developed countries) appear to dominate. Different localities also report differing frequencies of qualitative (i.e., type 2) VWD, each differentially ranging from 3% to >50% of all VWD cases. The frequency of specific qualitative VWD types (i.e., 2A, 2B, 2M and 2N) also differ. Importantly, the proportion of type 2 VWD cases is relatively low in developing countries, but this is unlikely to reflect true geographical variation, and instead more likely reflects usage of inadequate test panels that cannot distinguish these qualitative disorders from type 1 VWD. Similarly, considerable variance in identified type 2 VWD cases also exists among centers in developed countries, presumably also related to ineffective phonotypic test panels. For example, whereas 2A VWD is most often reported as the most prevalent type 2 VWD disorder, some centers reported correspondingly higher levels of 2M VWD [2,46,47]. This is most likely due to failure by most laboratories to properly discriminate types 2A and 2M VWD without a full test panel.

Management of VWD

The management of VWD has recently been extensively reviewed [48], so only a summary is presented here.

■ 'Standard therapy'

Therapeutic treatment for VWD primarily aims to 'replace' the VWF deficiency in order to prevent, arrest or manage bleeding. In general, this is mostly accomplished by desmopressin treatment and/or factor replacement therapy. As noted previously, desmopressin acts to release VWF stored within the patient's own endothelial cells. However, only some cases of VWD (i.e., those with mild quantitative deficiencies or mild qualitative defects), are responsive to desmopressin, since this agent only facilitates release of VWF that has already been produced and stored. The desmopressin response is also short lived, and tachyphylaxis leads to limited ongoing efficacy with short-term repeated treatments. In brief, desmopressin is effective for the short-term treatment of most patients with mild type 1 VWD, and some with type 2 VWD [42]. Desmopressin is ineffective in type 3 VWD, ineffective or insufficiently effective in many cases of type 2 VWD, and insufficiently effective for long-term treatment, or perioperative management of patients undergoing major surgery. Desmopressin is generally safe and well tolerated, but some side effects can be observed (e.g., headache, flushing, nausea, seizures and hyponatremia), and special care must be taken because of fluid retention. Desmopressin is not utilized by some clinicians in pregnancy [49–52] or in 2B VWD [51,53], although some

Table 4. Main differences and implications between factor concentrates.	
Main differences	**Implications/considerations**
Most concentrates contain both VWF and FVIII, but may differ widely in regards to their relative proportions	Implications in the context of therapeutic management. For example, concentrates with a high relative level of FVIII should be used with care to avoid increasing plasma FVIII to levels considered as posing a potential prothrombotic risk. Those with very low levels of FVIII may not provide sufficiently quick rises in FVIII:C for acute bleeds (e.g., in type 3 VWD)
Specific VWF activities or relative retention or loss of HMW VWF may vary broadly.	Since the HMW VWF forms define the most adhesive or functional forms of VWF, this would theoretically affect hemostatic efficacy. It is similarly worth considering the level of 'specific' VWF activity, as reflected by RCo/Ag or CB/Ag ratios
Safety processing (i.e., selective treatments for removal and destruction of potentially infectious agents such as HIV or hepatitis viruses)	Theoretical differences in infection risk. However, there has not been any recent case of HIV or hepatitis infection resulting from the use of any of the modern formulations to the best of the author's knowledge

CB/Ag: VWF collagen binding to antigen ratio; HMW: High-molecular weight; FVIII:C: FVIII coagulant; RCo/Ag: VWF ristocetin cofactor to antigen ratio; VWD: von Willebrand disease.

expert recommendations indicate that some of these cases can be treated (cautiously or selectively) with desmopressin and some centers have also used it safely in these settings [17,54–57].

Factor replacement therapy is the mainstay of biological therapy where desmopressin is not effective or not sufficiently effective [48]. This includes those patients who may be desmopressin responsive, but require long-term therapy. A wide range of concentrates are currently available worldwide [58,59], and there are several notable differential features worth mentioning (Table 4).

Interestingly, although concentrates are used primarily for VWF replacement, they are often only labeled with FVIII levels, dosed according to the level of FVIII and/or monitored by FVIII:C testing [48]. This sometimes relates to regulatory restrictions that prevent labeling of products with VWF activity levels or with more modern VWF activity parameters such as VWF:CB [5,58]. The resulting issue with this is that VWF and FVIII levels actually vary considerably within the different concentrates [58–60]. Thus, whilst a FVIII level post-concentrate treatment with one concentrate can be correlated to the VWF level or activity in that specific concentrate formulation, it will not reflect the same VWF level or activity within another concentrate for the same given level of FVIII. Moreover, in type 3 VWD, the level of FVIII will rise not only from the FVIII provided by the concentrate, but because of increasing stability of endogenous FVIII resulting from the infused VWF.

Concentrates are applied intravenously according to the need. Most national and expert guidelines will provide guidance on this, although there are some differences between recommendations [48,54,60,61]. Concentrate efficacy is monitored clinically (e.g., for signs of breakthrough bleeding) and using the same laboratory tests as defined earlier to diagnose VWD. In brief, and as partly explained above, this usually involves FVIII:C testing, but should also evaluate VWF level and activity. The latter is usually by VWF:RCo testing, although the use of the VWF:CB assay may also have some utility [19].

■ **Additional biological therapies in VWD**
A range of additional therapies is used in treating VWD, depending on geographic locality and on a case-by-case basis [2,48]. These include antifibrinolytic agents (e.g., tranexamic acid and aminocaproic acid; used for treatment of mucocutaneous bleeds, dental surgery and menorrhagia) and hormonal treatments (effectively helps manage menorrhagia in some cases). Additional treatments may be applied in specific cases or in developing countries where desmopressin and/or concentrates are unavailable [2,47–57,61–69].

■ **Global perspective**
Differential management for VWD is applied on the basis of both geographical and economic considerations. Standard therapy is used in most developed countries; desmopressin is employed wherever possible, and VWF(/FVIII) concentrates and antifibrinolytic therapy in other situations or as required. However, in developing countries treatment choices are often limited

or suboptimal. For example, VWF factor concentrate is limited in availability and/or comparatively very expensive and thus is used very sparingly in India and China, and desmopressin is often not available; thus, alternate treatment strategies need to be applied [49,62,63]. Regulatory considerations also influence geographic-based therapies in VWD. For example, although desmopressin is available in Australia, the nasal applied version is not. Although antifibrinolytic therapy is available in most geographies, the type available (i.e, tranexamic acid vs aminocaproic acid) will depend on regulatory clearances.

Future perspective
■ Diagnosis of VWD
General observations
It is difficult to accurately predict the future approaches to laboratory diagnosis of VWD, since this is often driven by pragmatism ahead of science or clear clinical utility. For example, pressures on laboratories to cut costs and improve turnaround times will see a decline in utility of some methodologies, even those with proven clinical utility, simply because laboratories will chose simpler or automated (but not necessarily more clinically useful) technologies. To a large extent, the adoption of laboratory tests in a given geographical boundary also depends on methodologies that have been cleared by regulatory bodies, again potentially 'promoting' assay usage on the basis of availability rather than on the basis of published evidence and clinical utility. This in turn may in part also depend on economic considerations, and the ability of commercial manufacturers to apply for and facilitate clearance of their tests ('*in vitro* diagnostics') through the regulators.

For example, a commercial monoclonal antibody-based assay performed by latex immunoassay (LIA) technology, using automated instrumentation, and marketed as a VWF:Act assay (Table 1) is cleared for laboratory use in the USA by the US FDA and is becoming increasingly popular in laboratories. This progression occurs because the assay is regulatory cleared for use (therefore laboratories are 'permitted' to use it in diagnostic testing), because the assay is easy to perform (fully automated, load and run), and because the assay otherwise behaves well in terms of standard laboratory parameters (e.g., precision and accuracy). The assay is also increasingly being adopted by laboratories in place of other VWF activity assays, notably the VWF:RCo assay, which (albeit with proven clinical utility in VWD diagnostics) suffers from poor precision and accuracy and is sometimes cumbersome and time consuming to perform. Nevertheless, the VWF:Act assay is not a true activity assay, and reflects

a test result that is distinct from the VWF:RCo assay result; hence, the replacement of the VWF:RCo assay by the VWF:Act assay in laboratories is a pragmatic decision – it will satisfy regulatory and accreditation requirements – but is not based on good scientific or clinical evidence of any comparability. Indeed, there is considerable evidence in the literature that the VWF:CB assay reflects better clinical utility for VWD than the VWF:Act assay [5,10,38–40]; however, as an FDA regulatory cleared VWF:CB assay does not exist, laboratories cannot use it to diagnose VWD in the USA. Moreover, the VWF:RCo and VWF:CB assays are generally more able to recognize the loss of HMW VWF than the VWF:Act assay [48], and loss of HMW VWF is a characteristic feature of many types of VWD (notably, 2A, 2B and PT-VWD).

There are several 'new' VWF:RCo-like assays in the pipeline [70,71], notably using recombinant forms of GPIBA, but independent validation is currently lacking. Additional improvements in the near future in VWD diagnosis will come from improvements to, or automation of, existing assays, as well as expanding test panels used by laboratories. The following section expands on this theme (Table 1).

FVIII:C assays
Most laboratories employ a one-stage clot-based assay. This is unlikely to change in the near future. Although there has been some regulatory push towards the use of chromogenic FVIII assays in the context of factor concentrate production and testing, this has not been translated into routine laboratory practice, even within hemophilia centers assessing factor concentrate therapy [72]. The one-stage clot-based assay is generally easier and cheaper for laboratories to perform, and given the lack of evidence base for substantive beneficial use of the chromogenic assay, the latter will remain a supplementary option.

VWF:Ag assays
VWF:Ag assays remain a core laboratory test, but there will be a continued movement away from ELISA-based and towards LIA-based technologies. This is simply a pragmatic decision; ELISA assays work well, and are generally better performing at low levels of VWF than LIA assays [33]. However, LIA assays are generally easier for most laboratories to perform, given that they can be performed on most current hemostasis analyzers.

VWF:RCo assays
The VWF:RCo assay were originally performed as a manual semi-quantitative assay using glass slides, later adapted to use on platelet aggregometers as a

semi-automated assay, and more recently adopted to use on automated analyzers [9]. There is an ongoing trend away from the use of aggregometers to that of automated analyzers, and there are many publications indicating successful implementation on many analyzers [9]. Nevertheless, whilst automated analyzers tend to provide better assay precision, aggregometry-based assays usually have better lower limit of VWF detection [33], which is important in VWD diagnostics given that most severe cases of VWD have very low VWF:RCo levels. However, this lower limit of VWF detection can be easily improved with assay modification [73,74]. There have been several attempts to move VWF:RCo assays to alternate methodologies, including ELISA and flow cytometry [9]; however, these have yet to capture the attention of laboratory practitioners. More recently, as noted above, automated VWF:RCo-like assays [71] have been described that do not employ ristocetin to facilitate VWF-mediated agglutination. These can be automated to modern hemostasis analyzers. Preliminary data looks promising, but the assay requires independent validation.

VWF:CB assays

Owing to published evidence of clinical diagnostic utility in VWD [5,10,36–40], this assay will continue to gain increased acceptance in VWD diagnostics, and may eventually be adopted to LIA technology to facilitate improved laboratory take up. An increasing number of commercial (ELISA-based) options are also becoming available [36], which is a reassuring sign of growing acceptance. The lack of a regulatory-cleared VWF:CB assay, however, will delay its uptake in North America, which is a notable problem in facilitating improved VWD diagnostics in that region [39–41]. Also important is the need to ensure that laboratories employ well standardized and HMW VWF-sensitive and optimized assays. There are, for example, a large number of commercial assays in the marketplace, and these differ

in HMW VWF sensitivity and thus in their ability to differentiate types 1 and 2 VWD [11,36].

VWF multimer assays

VWF multimer assays are poorly performed by most laboratories [37,38]. Despite recent reported improvements to methodology, the complexity and time-consuming nature of the test would suggest its continued decline in future usage.

VWF:pp assays

The VWF:pp assay [43] will likely gain more widespread acceptance over time, but continued take-up awaits
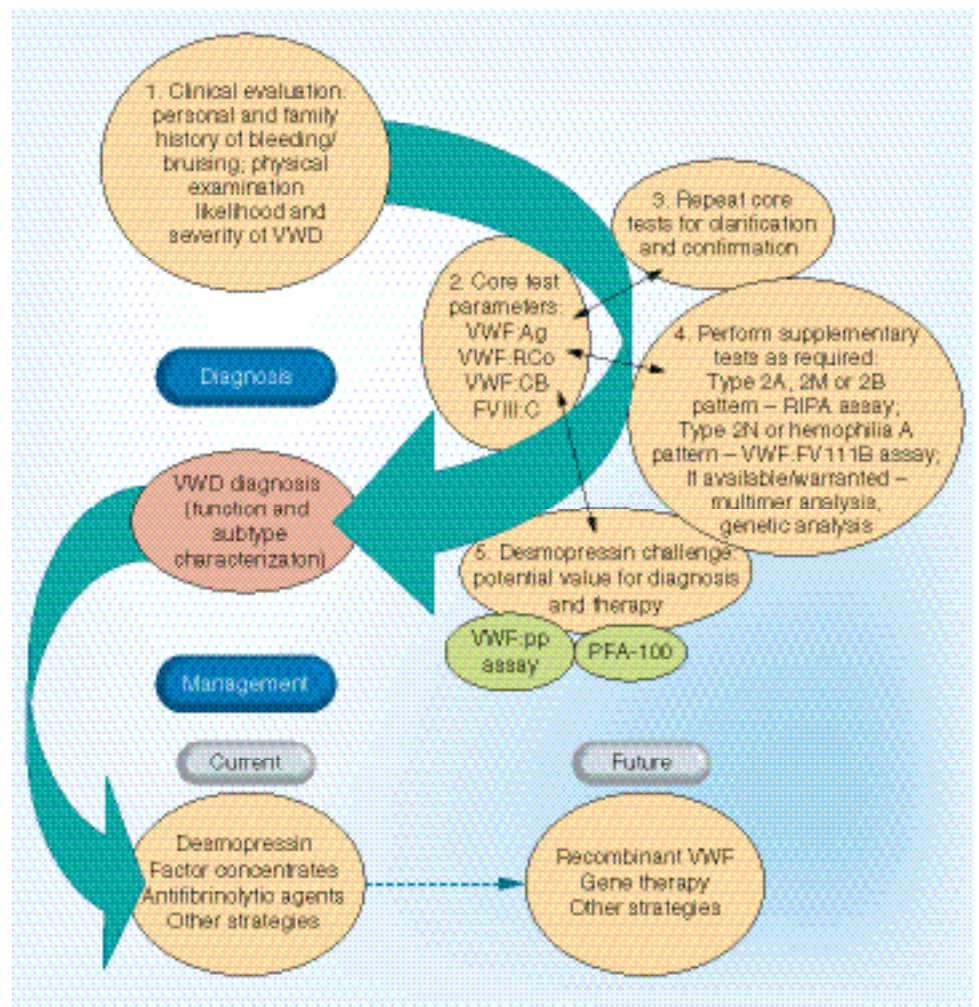
Figure 1. A progressive process for the characterization of individuals with von Willebrand disease and then their therapeutic treatment. With each step comes an increasing diagnostic certainty, and a more comprehensive characterization of that individual's VWD. The type of therapy is partly dependent on an appropriate diagnosis and VWD-type identification.
FVIII:C: FVIII coagulant; RIPA: Ristocetin-induced platelet agglutination; VWD: von Willebrand disease; VWF:Ag: VWF antigen; VWF:CB: VWF collagen binding; VWF:FVIIIB: VWF:FVIII binding; VWF:pp: VWF propeptide; VWF:RCo: VWF ristocetin cofactor.
Modified with permission from [4].

further evidence of clinical utility as well as improved (automatable) technology.

PFA-100

The PFA-100 has recently been given a new test cartridge and also remodeled into the PFA-200; however, these changes will not likely alter utility for VWD. Nevertheless, the potential utility of the PFA-100 in VWD as related to differential desmopressin responses in different VWD types [19] may lead to better utilization by laboratorians and clinicians alike.

■ Novel & future therapeutic approaches
Prophylaxis in VWD

The concept of prophylaxis in hemophilia is now firmly accepted as the gold standard in treatment modality for severe hemophilia [75]. By contrast, the concept of prophylaxis in severe VWD seems to be taking longer to develop [48,76,77]. However, patients with severe forms of VWD may have frequent hemarthroses and thus develop target joints similarly to patients with severe hemophilia, suggesting that secondary long-term prophylaxis with factor concentrates (rather than on-demand treatment on the occasion of their bleeding episodes) should similarly be considered.

■ Recombinant VWF

For several years, recombinant FVIII and FIX have represented the mainstay of treatment of hemophilia A and B, respectively [78,79]. Recombinant VWF for use in VWD was in fact developed over a decade ago [80], but has only recently been reinvestigated and entered into clinical trials [81,82,101]. Nevertheless, depending on successful human trials, recombinant VWF has the potential to alter the VWD treatment landscape in a similar way to that of recombinant FVIII and FIX.

■ Gene therapy & alternate emerging therapies for VWD

Several alternative approaches are being trialed to complement existing therapies for VWD, such as inducing endogenous expression with IL-11 or introducing the protein via gene delivery [83]. Gene therapy has been trialed with varying success in hemostasis disorders, most notably hemophilia A and B [84–87]. Gene therapy has the potential for long-term – if not lifelong – correction of VWD with abrogation of the need of future prophylaxis or repetitive, on-demand replacement therapy and improved quality of life. However, despite successful animal studies [88,89], trials in humans are lacking. Of greater challenge to VWF gene therapy, as compared with hemophilia, is the high complexity of VWF biosynthesis, which requires an efficient incorporation of this large protein into integrating (e.g., viral) vectors, as well as to complex intracellular (e.g., multimerization)

Executive summary

Background
- Von Willebrand disease (VWD) is considered to be the most common inherited bleeding disorder.
- VWD is due to deficiencies and/or defects in VWF, an adhesive plasma protein that binds to and stabilizes FVIII function, and that also facilitates binding of platelets to each other and to subendothelium.
- Deficiencies of VWF or defects in these or other functions of VWF, means that VWD is not a discrete disorder, but represents complex and diverse conditions to diagnose and manage.
- A battery or large panel of laboratory tests is required to appropriately diagnose or exclude VWD.

Global perspectives in diagnosis & prevalence of VWD & VWD types
- Different prevalence rates have been ascribed for VWD as well as its many types in different geographies.
- This is due to many factors, for example: consanguinity in developing countries leads to increases in familial and type 3 VWD; definitions of 'VWD' (e.g., inclusion or exclusion of cases of 'mild' or 'possible' type 1 VWD); issues related to laboratory testing (e.g., limited test panels or investigation of only the most severe bleeding problems in developing countries).

Management of VWD
- For treatment of VWD, most developed countries currently use standard therapy to manage bleeding, employing desmopressin wherever possible, factor concentrate in other situations and additional (e.g., antifibrinolytic) therapy when required.
- However, there are differences in content between factor concentrates, in relation to levels and composition of VWF and FVIII, and only selective concentrates are available in different geographic localities.
- These factors compromise optimal clinical management using replacement therapy, or attempts to follow expert guidelines related to diagnosis and management.

Future perspective
- Development of new tests and improvements to existing tests will continue to streamline and improve VWD diagnosis.
- Recombinant VWF has been developed and is undergoing clinical trials, and this promising therapy may change the VWD management landscape in the near future.

and extracellular (e.g., ADAMTS13 digestion) processing steps to generate a fully functional protein.

Conclusion

This report provides an overview of current 'standard' practice as applied to the diagnosis and management of VWD, as well as a possible future. In order to achieve an optimal diagnosis and management of people affected by VWD, several considerations are needed. This includes providing the best tests, methodologies and test panels available, and applying these appropriately within a well-considered diagnostic strategy. The availability and application of optimized treatment options is also important, and many of these considerations are dictated by economic and regulatory factors. Global improvements in both the diagnosis and management of this common bleeding disorder are likely in the next 5 years. Figure 1 provides a summary of these considerations.

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