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GUIDELINE

BSH Guideline

Guideline for laboratory diagnosis and monitoring of von Willebrand disease: A joint guideline from the United Kingdom Haemophilia Centre Doctors' Organisation and the British Society for Haematology

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SUMMARY

This guideline updates the previous guidelines[1,2] published on behalf of the British Society for Haematology (BSH) and the United Kingdom Haemophilia Centre Doctors' Organisation (UKHCDO), focussing on the laboratory components of diagnosis and monitoring. Clinical aspects will be addressed in a separate guideline.

METHODOLOGY

This guideline was compiled according to the BSH process at https://b-s-h.org.uk/media/16732/bsh-guidance-development-process-dec-5-18.pdf. The writing group, which comprised selected members of the BSH Haemostasis and Thrombosis Task Force (BSH HTTF), the UKHCDO Laboratory Working Party (LWP) and members of the UKHCDO Genetics Laboratory Network (GLN), produced the first draft of the manuscript. A literature search was carried out using the terms given in Table S1. The Grading of Recommendations Assessment, Development and Evaluation (GRADE) nomenclature was used to evaluate the levels of evidence and to assess the strength of recommendations. The GRADE criteria can be found at http://www.gradeworkinggroup.org and is summarised in appendix 3 of the guidance document linked above.

Review of the manuscript

Review of the manuscript was performed by the BSH HTTF, the BSH Guidelines Committee and the sounding board of BSH. It was circulated to members of the UKHCDO LWP and GLN, and was on the members section of the BSH website for comment.

GUIDELINE UPDATE

This guideline describes laboratory tests used to diagnose and monitor individuals with von Willebrand disease (VWD).

Since the publication of the previous guideline,[1] new functional tests have become widely available as an alternative to the ristocetin cofactor assay. This guideline also updates genetic testing rationale and highlights the American College of Medical Genetics and Genomics (ACMG) guidelines for interpretation of sequence variants[3,4] and Association for Clinical Genomic Science (ACGS) guidelines for variant interpretation.[5]

BACKGROUND

von Willebrand factor (VWF) is a large complex plasma glycoprotein essential for normal haemostasis. A reduction in VWF results in a bleeding disorder, as a quantitative defect in type 1 or type 3 VWD, or as a qualitative defect in type 2 VWD, or in acquired von Willebrand syndrome (AVWS). This varies in severity according to the degree of deficiency and the characteristics of VWF.

The complex structure of VWF and the wide range of plasma VWF levels, in the normal population, during pregnancy, in acute illness and after exercise, make laboratory assessment and diagnosis challenging.

A reduced VWF activity (<30 IU/dL) is usually associated with bleeding symptoms and is likely to be associated with a variant in the *VWF* gene, but these associations are less strong for reduced VWF activities between 30 and 50 IU/dL.[1] VWF activity between 30 and 50 IU/dL in isolation may be insufficient to result in significant bleeding, although some individuals with VWF activity between 30 and 50 IU/dL do have significant bleeding symptoms: this is likely to reflect the interaction with additional abnormalities in the haemostatic pathway, including mild platelet defects.[1]

Caution should be exercised in diagnosing VWD in individuals with VWF between 30 and 50 IU/dL to avoid the burden of an unnecessary diagnosis and the hazard of failing to complete further investigations, but some of these individuals may have bleeding for which lack of VWF activity is the primary cause.

VWF levels are known to increase with age[6]: VWF levels below 50 IU/dL normalise in approximately 43% of individuals.[7] Levels increase in individuals with type 1 VWD, whereas in those with type 2 VWD, activity levels do not increase.[8]

Pre- and postanalytical considerations

Preanalytical issues affect the quality of test results[9] for the diagnosis and monitoring of VWD, and are responsible for more than 70% of laboratory errors.[10] With specialist coagulation testing often performed at regional facilities in the United Kingdom, such errors can also occur in the local laboratory prior to sample transport.

Clinical requesting and sample collection

VWF levels may be elevated during inflammation, after exercise, postoperatively and during pregnancy, potentially masking VWD.[9] The collection of samples for diagnosis should be avoided at these times, and any diagnosis of VWD must be made on samples collected on two separate occasions.

Detailed descriptions of sample handling for coagulation assays can be found in recent guidelines from the BSH[11] and the International Council for Standardization in Haematology.[12]

Sample handling/transportation

Samples should be collected into 3.2% sodium citrate and transported to the laboratory as whole blood at ambient temperature (18–25°C).[11,12] Transportation of whole blood samples on ice leads to the precipitation of VWF and factor VIII (FVIII),[13] and the storage of whole blood samples at 2–8°C can lead to time-dependent reduction in VWF and FVIII activity (FVIII:C).[9,14,15] This results in a significant risk of normal individuals being misdiagnosed as having type 1 VWD, and of individuals with type 1 VWD being misdiagnosed as having a type 2 VWD.[13,16]

Whole blood samples at ambient temperature are stable for 24–28 h for an activated partial thromboplastin time (APTT), VWF antigen (VWF:Ag) and VWF activity measured by the ristocetin cofactor assay (VWF:RCo), but only for 8–12 h for FVIII:C.[17] Therefore whole blood samples for assays for VWD should reach the laboratory as soon as possible, and not more than 12 h, after sample collection.

Clotted samples, and those that are under-filled or overfilled, should be rejected for analysis.[11]

Sample processing

Samples should be centrifuged at 18–25°C for 10 min at 1500–2000 g in a centrifuge that has a rotor with swing-out buckets.[11] Stability data for citrated plasma that has been separated from blood cells show that FVIII:C reduces by up to 14.8% (depending on the reagent used) in 4 h after plasma separation.[18] Therefore, plasma should be separated from cells and testing completed within 4 h of separation, and if testing cannot be completed within that time frame, plasma should be frozen for future testing.

Although there are few data on the effects of haemolysis, icterus or lipaemia on VWF assays, results may be affected and plasma should be visually examined before testing.[9–12] Haemolysis may lead to unpredictable effects in routine assays, depending on the mechanism and extent of haemolysis: in the absence of data to suggest otherwise, samples should be rejected and re-collected, unless in vivo haemolysis is suspected. Icterus may interfere with assays that measure optical density, especially if the assay uses a chromophore that has a similar colour to bilirubin: these interferences are assay specific and affected assays should not be performed when the interference is clinically significant. Lipaemia may cause issues with assays that measure optical density, especially in latex immunoassays. These interferences are assay specific and affected assays

should not be performed when the interference would be clinically significant. High-speed centrifugation can be considered to remove the lipid layer prior to sample freezing.[19]

For plasma that is to be frozen, the sample should be stored in a screw cap polypropylene tube with an 'O'-ring. Samples should be stored below −70°C in freezers without auto-defrost cycles, although storage below −24°C can be used for short periods (up to 3 months).[20] Frozen plasma shipped to another laboratory should be sent on dry ice to ensure that it remains frozen.[11]

Frozen plasma must not be allowed to thaw at room temperature (to avoid precipitation of VWF and FVIII), but should be thawed in a temperature-controlled water bath at 37°C with the surface of the frozen plasma at or below the surface of the water.[12] For plasma volumes less than 1 mL, a thaw of 5 min is sufficient, although the time can be reduced if the sample is completely thawed before this time.[11,12,21,22] Samples should be mixed thoroughly by inversion prior to testing,[11] as inadequate mixing can lead to a false VWD diagnosis.[12,21] In one study (using one reagent/analyser combination), repeated freeze–thaw cycles reduced the median result by more than would be expected by interassay variation for FVIII:C after three thaw cycles (−6.0% variation) and VWF:Ag after six cycles (−9.6% variation) but not for VWF:RCo (−0.8% after seven cycles)[22]; FVIII:C and VWF antigen and activity assays should not be performed on plasma aliquots that have already been through a freeze–thaw cycle, unless local verification of these findings shows otherwise.

Interlaboratory variations

Assays for VWF and FVIII:C are particularly sensitive to differences in sample handling. One multicentre retrospective study showed frequent low VWF results in local laboratories that were not replicated when the individuals were retested at a specialist centre.[23]

A recent survey of participants in an international external quality assurance (EQA) scheme showed that a type 1 VWD sample with a median VWF:RCo of 28 IU/dL was correctly classified by 94% of participants, but a type 1 VWD sample with a median VWF:RCo of 15 IU/dL sample was only correctly classified by 78% of participants.[24]

To minimise these differences, consideration should be given to testing of samples at centres with expertise in performing the assays and interpreting the results, especially when confirming a diagnosis; collection of the samples at the site of testing should also be considered.

Intralaboratory and intraindividual variations

Diagnosis of VWD/AVWS should be confirmed by testing on two occasions, but it should be noted that both intralaboratory imprecision and intraindividual variation have the potential to give false-normal results and false-abnormal results. However, studies have shown that a VWF antigen of >100 IU/dL excludes VWD on a single test with a negative predictive value of between 89%[25] and 95%.[26]

Reference ranges

Although decisions on the diagnosis of VWD are based on specific cut-offs of 30 or 50 IU/dL,[1] it is a requirement of ISO15189[27] to quote reference ranges with all examinations where possible. For VWF assays, reference ranges should be locally determined from 120 healthy donors using collection, processing and analysis techniques that are identical to those used for patient samples.[11] Thus, if the laboratory primarily analyses frozen and thawed plasma, rather than fresh plasma, then reference ranges should be established with frozen plasma from normal donors. An alternative approach is to verify a manufacturer's range by analysis of 20–40 such plasmas and checking for concordance.[11]

Accreditation and quality assurance

Diagnostic and genetic laboratories should be accredited to ISO15189,[27] which in the United Kingdom is overseen by the United Kingdom Accreditation Service.

ISO15189 includes a requirement to participate in an accredited external quality assurance program, and these are available from UK National External Quality Assessment Scheme for Blood Coagulation for diagnostic and genetic laboratories, and Genomics Quality Assessment for genetics laboratories, among others. These cover all aspects of the diagnostic process from phenotypic testing to nucleic acid extraction and genetic analysis, to the description and classification of the variant(s) detected, and the production of an interpretative report.

Recommendations

- Whole blood samples for coagulation tests should be maintained at an ambient temperature of 18–25°C during transport and storage prior to processing, and should not be refrigerated (1C).
- The time between sample collection and testing (or freezing) of citrated plasma for VWD assays should be less than 12 h (1C).
- Before a diagnosis of VWD/AVWS is made, we suggest that abnormal results are confirmed at a specialist centre with experience of performing the assays and interpreting the results, and that samples are collected on site (2D).

Laboratory investigations

A flow chart for the laboratory investigation of suspected VWD and/or AVWS can be seen in Figure 1.

Full blood count

In all cases of suspected VWD/AVWS, a full blood count (FBC) should be performed to count platelets and measure mean platelet volume (MPV). Platelet numbers and size are generally normal in all types of VWD, but in type 2B VWD individuals may have a mild thrombocytopenia or a normal platelet count with either normal-sized platelets[28] or giant platelets.[29] In platelet-type (pseudo) VWD (PT-VWD), individuals generally have a macrothrombocytopenia of varying degrees.[30] Some individuals with essential thrombocythaemia (or other myeloproliferative neoplasms) may develop AVWS.[31]

A full initial investigation for suspected VWD/AVWS should be performed regardless of the platelet count.

Activated partial thromboplastin time

A coagulation screen including prothrombin time (PT), APTT and Clauss fibrinogen assay should be performed on individuals being investigated for a bleeding tendency.

The laboratory reagent for APTT should be sensitive to FVIII:C<30 IU/dL[32]: Many common reagents are suitably sensitive, but not all,[33] and one study showed that a normal APTT could be seen in patient samples with FVIII:C as low as 12 IU/dL.[34]

Normal APTT results do not exclude a diagnosis of VWD/AVWS. A full initial investigation for suspected VWD/AVWS should be performed regardless of the APTT.

Platelet function Analyser (PFA-100/200)

The PFA-100/200 (PFA) analyser (Siemens, Germany) provides an in vitro assessment of some components of primary haemostasis under high shear conditions. Although the PFA cannot be recommended for the diagnosis of non-severe platelet disorders,[35,36] it may play a role in the diagnosis of VWD. The PFA sensitivity to VWD is 85%–90%, with almost 100% sensitivity to type 3, type 2A and PT-VWD.[36] However, in type 1 VWD the PFA is normal in some cases with VWF levels <25 IU/dL,[36] and can be normal or abnormal in individuals with low VWF.[37]

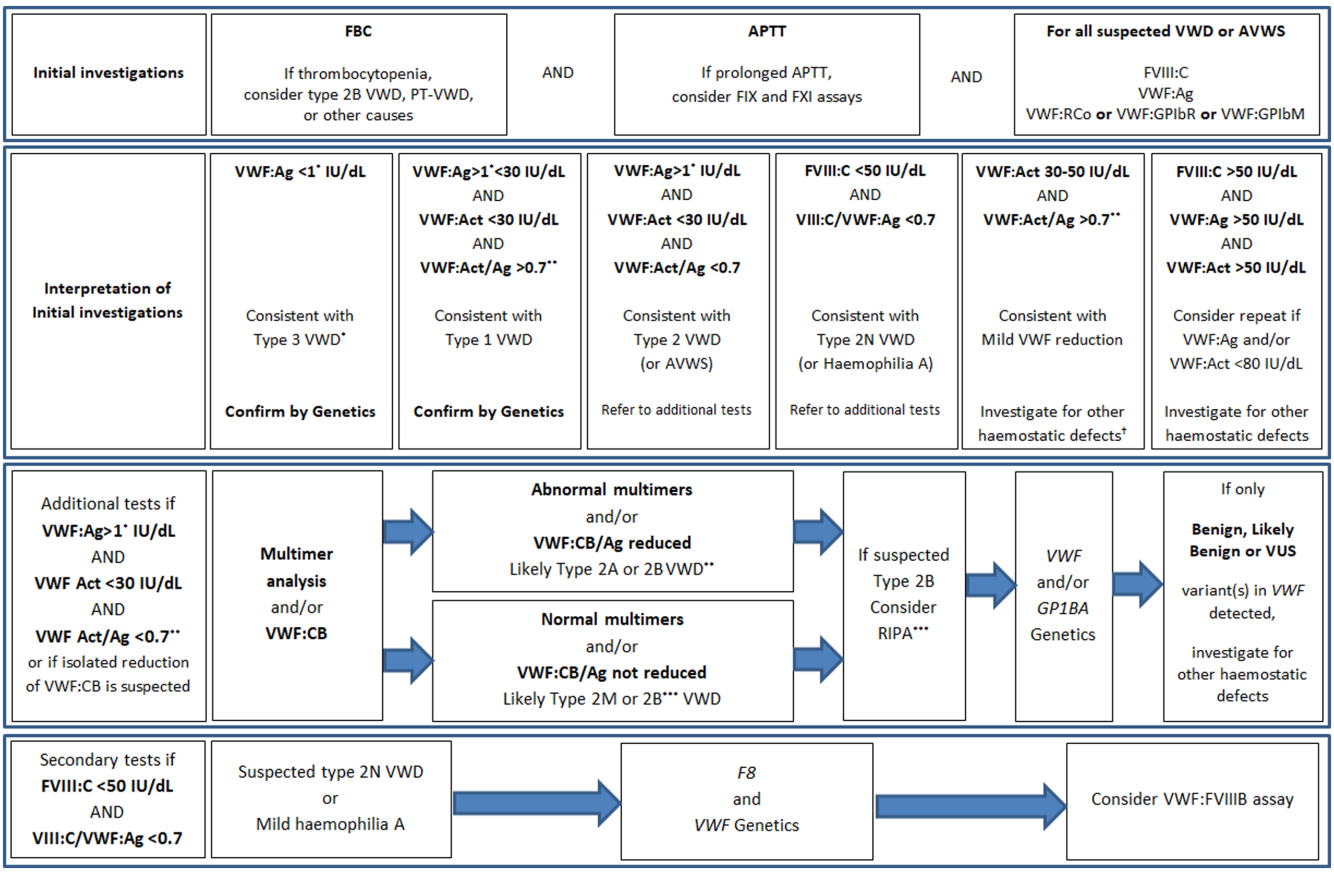

FIGURE 1 Algorithm for laboratory diagnosis of von Willebrand disease and acquired von Willebrand syndrome. *Laboratories using a VWF:Ag assay with lower limit of quantitation above 1 IU/dL should be aware that type 3 VWD cannot be differentiated from severe type 1 VWD and genetics should be performed. **Individuals with rare variants that affect only the ability of VWF to bind collagen will have a normal VWF:Act/Ag but a reduced VWF:CB/Ag ratio. ***Multimers are normal in some individuals with type 2B—RIPA should be considered if there is concern about a type 2B VWD regardless of multimer or VWF:CB results. † Some individuals with mild VWF reduction may have VWD. APTT—activated partial thromboplastin time; AVWS—acquired von Willebrand Syndrome; FBC—full blood count (including MPV); FIX—factor IX; FVIII:C—factor VIII activity assay (one-stage or chromogenic); FXI—factor XI; PT-VWD—platelet-type (pseudo) von Willebrand Disease; RIPA—ristocetin induced platelet agglutination assay; VIII:C/VWF:Ag—factor VIII activity to VWF antigen ratio; VWD—von Willebrand Disease; VWF—von Willebrand factor; VWF:Act—VWF activity by VWF:RCo, VWF: GPIbR or VWF:GPIbM assay; VWF:Act/Ag -VWF:RCo, VWF:GPIbR or VWF:GPIbM activity-to-antigen ratio; VWF:CB/Ag—VWF:CB activity-to-antigen ratio.

The PFA is twice as likely to be abnormal in individuals with platelet count $<150 \times 10^9$/L compared to those with platelet count $>150 \times 10^9$/L,[38] and will also be abnormal in those with a low haematocrit (<0.25).[39]

A full initial investigation for suspected VWD/AVWS should be performed regardless of the results of a PFA screen.

Bleeding time

The skin bleeding time should not be used as it is poorly standardised and poorly reproducible.[40,41]

Blood group

While individuals with blood group O have up to 25% lower levels of VWF than non-O individuals, and therefore are more likely to have a diagnosis of type 1 VWD made,[6] they are no more likely to inherit type 2 or type 3 VWD, and the bleeding phenotype of individuals with VWD is the same regardless of blood group. Therefore, there is no need for blood group-specific reference ranges,[7] nor to check blood group for VWD diagnosis.

Recommendations

- A FBC (including mean platelet volume) with a blood film for platelet morphology should be performed on all individuals being investigated for VWD/AVWS (1B).
- A PT, APTT and Clauss fibrinogen assay should be performed on all individuals being investigated for VWD/AVWS (1B).
- A PFA-100/200 analyser should not be used to screen for VWD/AVWS (1B).
- Blood group-specific reference ranges for the diagnosis of VWD should not be used (2C).

INITIAL TESTS USED FOR THE DIAGNOSIS OF VWD/AVWS

Measurement of FVIII:C

FVIII:C should be measured in all individuals suspected of having VWD or AVWS. This can be measured by a one-stage clotting assay (OSCA) or chromogenic substrate assay (CSA) and numerous guidelines are available for best practice.[7,11]

A VWD/AVWS diagnosis may be missed if the clinical request is for FVIII:C only,[9,10] so a FVIII:C assay alone is not enough to make or exclude a diagnosis of VWD/AVWS.

Assays of VWF antigen

VWF:Ag should be measured in all individuals suspected of VWD or AVWS. Levels are quantified by immunological methods that include enzyme-linked immunosorbent assays (ELISAs), automated immunoturbidometric methods utilising latex particle agglutination (LIA) and more recently by chemiluminescent immunoassay (CLIA) methodology.[42] Limitations of LIA and CLIA assays include the presence of interfering factors such as high rheumatoid factor levels or heterophile antibodies that can falsely elevate levels.[1] Furthermore, the lower level of detection and the lower limit of quantification (LLoQ) vary between assays and should be verified prior to use in each laboratory.[11] Many LIA VWF:Ag methods cannot easily discriminate severe type 1 VWD from type 3 VWD due to lack of sensitivity below 2–3 IU/dL. These include the HemosIL von Willebrand Factor Antigen,[43] Hyphen LIAPHEN vWF-Ag,[44] Siemens VWF Ag[45–47] and Stago STA-Liatest VWF:Ag[48] assays.

VWF:Ag assays based on flow cytometric techniques have not yet been proven to be as sensitive as automated assays.[49] Pending further data, they cannot be recommended for use in the diagnosis of VWD.

For the diagnosis of type 3 VWD, the assay used for measuring VWF:Ag should be demonstrably capable of measuring to <1 IU/dL. If the VWF:Ag assay used has LLoQ >1 IU/dL, laboratories should be aware that severe type 1 and type 3 VWD cannot be differentiated by that method and results should be interpreted accordingly.

Assays of VWF activity

Binding of VWF to platelet GPIb (VWF:RCo, VWF:GPIbM, VWF:GPIbR and VWF:Ab)

The platelet-based ristocetin-dependent assays for VWF activity[50] (VWF:RCo) are long-established as a measure of VWF function and for use in VWD diagnosis and classification. However, they are affected by poor standardisation, poor reproducibility and poor sensitivity at low levels (although these have been partially addressed by automated assays).[51–53] Alternative functional assays have been developed and automated during the last decade as surrogate measures of VWF-binding ability to platelet GPIb-V-IX, but, like VWF:RCo, they are not physiological. Assays contain either ristocetin plus wild-type recombinant GPIb-fragment or mutant recombinant GPIb-fragment containing two gain-of-function mutations with enhanced ability to bind VWF in the absence of ristocetin. An international nomenclature differentiates these assays[54] with the former being denoted VWF:GPIbR and the latter VWF:GPIbM. The current WHO 6th IS (07/316) international reference preparation for VWF in plasma has incorporated VWF:GPIbR and VWF:GPIbM into the value for VWF:RCo.[55]

Direct comparisons of these assays have highlighted differences in sensitivities between VWF:RCo, VWF:Ab, VWF:GPIbR and VWF:GPIbM assays which potentially alter typing and subtyping of VWD, particularly in types 2A or 2M VWD,[56–58] and particularly if the variant alters the reagent-binding characteristics. However, a recent

meta-analysis of published data showed comparable accuracy between VWF:RCo, VWF:GPIbR and VWF:GPIbM.[59]

VWF activity assays based on flow cytometric techniques have not yet been proven to be as sensitive as automated VWF:GPIbR assays.[49] Pending further data, they cannot be recommended for use in the diagnosis of VWD.

Laboratories reporting VWF activity assays should state clearly which type of assay has been performed, using the standard nomenclature.[54] Commonly used tests available in the United Kingdom with the correct assay nomenclature are shown in Table 1. A schematic diagram of the assays is shown in Figure S1.

TABLE 1 Commonly used assays in the United Kingdom and their nomenclature.[52]

Assay type	Reagent [technique]
VWF antigen (VWF:Ag)	Hyphen Biomed LIAPHEN VWF:Ag [LIA] Hyphen Biomed ZYMUTEST vWF [ELISA] Siemens vWF Ag [LIA] Stago Asserachrom VWF:Ag [ELISA] Stago STA Liatest VWF:Ag [LIA] Technoclone Technozym vWF:Ag [ELISA] Werfen AcuStar von Willebrand Factor: Ag [CLIA] Werfen HemosIL von Willebrand Factor Antigen [LIA]
VWF activity (VWF:RCo)	Helena Ristocetin Cofactor [agglutination] Siemens BC von Willebrand [agglutination] Stago STA VWF:RCo [agglutination]
VWF activity (VWF:GPIbR)	Werfen AcuStar von Willebrand Factor: RCo [CLIA] Werfen HemosIL VWF:RCo activity assay [LIA]
VWF activity (VWF:GPIbM)	Siemens INNOVANCE VWF:Ac [LIA]
VWF activity (VWF:CB)	Hyphen Biomed ZYMUTEST vWF:CBA [ELISA] Stago Asserachrom VWF:CB [ELISA] Technoclone Technozym vWF:CBA [ELISA] Technoclone Technozym vWF:CBA Collagen Type I [ELISA] Technoclone Technozym vWF:CBA Collagen Type VI [ELISA] Werfen AcuStar von Willebrand Factor: CB [CLIA]
VWF FVIII binding (VWF:FVIIIB)	Stago Asserachrom VWF:FVIIIB [ELISA]
VWF activity (VWF:Ab)	Werfen HemosIL VWF activity [LIA]

Abbreviations: CLIA, chemiluminescence assay; ELISA, enzyme-linked immunosorbent assay; LIA, latex immunoassay.

VWF:RCo

The VWF:RCo assay is sensitive to reduction in VWF ability to interact with platelet GPIb-V-IX, but is limited by high inter- and intra-assay variation and poor accuracy at low levels. Even with automated assays, the LLoQ for VWF:RCo is 3–10 IU/dL,[60,61] which is too high to reliably differentiate between severe type 1 and type 2 VWD from type 3 VWD.

Population-based studies have shown that VWF activity measured by VWF:RCo is reduced compared to levels of VWF antigen in individuals with the p.(Arg1472His)[62] or p.(Pro1467Ser)[63] variants; these variants are directly adjacent to a ristocetin-binding region in the VWF A1 domain, but are not associated with a bleeding phenotype.[62,63] In the 1000 Genomes project, the p.(Arg1472His) variant was found at a minor allele frequency (MAF) of 0.100 in Europeans, 0.108 in East Asians, 0.153 in South Asians and 0.551 in Africans[64] and is classified as benign/likely benign[65]; p.(Pro1467Ser) has a MAF of 0.00000657.[65]

VWF:GPIbR

VWF:GPIbR assays are based on the ability of VWF to bind recombinant wild-type GPIb-fragment in the presence of ristocetin, and are highly sensitive and moderately specific.[59] Some VWF:GPIbR activity assays are falsely reduced in individuals with p.(Arg1472His) and p.(Pro1467Ser),[58] but others are not.[57,58] Both the Werfen HemosIL VWF:RCo LIA and the Werfen AcuStar VWF:RCo CLIA measure VWF:GPIbR (not VWF:RCo); these assays have limitations associated with LIA and CLIA assays as previously described.

ELISA assays that use VWF:GPIbR produce significantly higher activities in individuals with type 2B VWD[58] and type 2M[66,67] and are not recommended for use.

VWF:GPIbM

VWF:GPIbM assays are based on the ability of VWF to bind a recombinant GPIb-fragment modified with two gain-of-function mutations, and are highly sensitive and moderately specific.[59] The assay does not utilise ristocetin so is insensitive to the p.(Arg1472His) and p.(Pro1467Ser) variants.[63] The automated Siemens INNOVANCE VWF:Ac LIA measures VWF:GPIbM and has limitations associated with LIA assays as previously described. Erroneously high (normal) VWF:GPIbM activities have been reported in an individual with AVWS with VWF:Ag 13 IU/dL[68] and in an individual with a diagnosis of type 3 VWD and VWF:Ag 2 IU/dL[69] due to the presence of an unspecified interfering substance, thought to be possible anti-mouse antibodies.

ELISA assays that use VWF:GPIbM produce significantly higher activities in individuals with type 2B VWD[58] and type 2M[65,66] and are not recommended for use.

VWF:Ab

Monoclonal VWF:Ab assays do not directly measure VWF function and consistently measure higher activities in individuals with type 2A VWD who have the p.(Val1665Glu)

variant,[58] and in type 2M VWD.[70] A recent EQA study[24] also showed that these assays were also more likely to cause a misclassification of type 1 VWD as type 2 VWD. The HemosIL VWF activity LIA measures VWF:Ab.

These assays are not recommended for use.

Activity-to-antigen ratios

VWF activity/VWF:Ag ratios (calculated to one decimal place) of <0.5 to <0.7[7] are recommended to indicate type 2A, 2B or 2M VWD, but the choice of a universal cut-off ratio is arbitrary and the use of a cut-off that is too low or too high may risk misclassifying some patients; it is likely that such cut-offs should be method specific.[71] A recent meta-analysis of studies examining VWF levels in the diagnosis of VWD has suggested that a cut-off of <0.7 is more appropriate than a cut-off of either <0.5 or <0.6.[72] It must be noted that in some individuals, reduced ratios can be calculated despite normal VWF activity and VWF:Ag levels; this has been reported in type 2B VWD[73] and AVWS.[74]

We suggest that if laboratories report activity-to-antigen ratios that they are reported without a reference range.

Recommendations

- In the initial investigation for VWD or AVWS, FVIII:C, VWF antigen and VWF activity (by VWF:RCo or VWF:GPIbR or VWF:GPIbM) should be measured (1B).
- Laboratories should calibrate assays using plasmas that are traceable to the International Standard for FVIII:C and for VWF, and results should be reported in IU/dL (1C).
- Additional testing for the classification of VWD should be performed in individuals with a VWF activity-to-antigen ratio of <0.7 (2B).
- Monoclonal VWF:Ab assays or flow cytometry assays to measure VWF activity are not currently recommended (1C).
- Laboratories diagnosing type 3 VWD should verify that methods employed to measure VWF:Ag are demonstrably capable of measuring to levels <1 IU/dL (1C).

ADDITIONAL TESTS USED FOR THE CLASSIFICATION OF VWD

VWF collagen-binding activity (VWF:CB)

The assessment of the ability of VWF to bind to different types of collagen can improve the diagnosis and subtyping of VWD,[75–77] and a rare subset of individuals with 2M VWD with reduced collagen binding but normal platelet-dependent binding can be misdiagnosed if only a platelet-dependent VWF activity assay is performed during the initial investigation for VWD.[76] However, in the experience of the writing group these defects are extremely rare, suggesting that VWF:CB assays do not need to be performed as part of the initial investigation for VWD diagnosis. The consequences of failing to detect these rare defects must be balanced against the impact on laboratories of providing the assays, including cost, training, equipment and compliance with ISO15189. It should also be noted that not all VWF:CB assays will detect all abnormalities of VWF:CB.[76] However, laboratories not performing VWF:CB assays should consider referring samples to another laboratory if no other cause for a bleeding diathesis is identified.

Binding to human type I and/or type III collagen is most commonly performed by ELISA or CLIA although assessment of binding to other collagen types may also aid diagnosis,[78,79] and CLIA assays have limitations as previously described. The use of VWF:CB assays has been demonstrated to be less sensitive than GPIb binding assays, with some individuals with type 2A VWD being classified as having type 1 VWD if the only functional assay performed was VWF:CB.[57,80]

VWF:CB assays are sensitive to a loss of high molecular weight multimers (HMWMs) and have been reported to correlate well with VWF:RCo assays in all but type 2 VWD.[81] A ratio of VWF:CB to VWF:Ag may be used cautiously as an alternative to multimer analysis,[7,82] but it must be noted that the use of VWF:CB/VWF:Ag ratios is less sensitive than multimer analysis in some individuals with VWD.[83]

Ratios of collagen binding to VWF antigen (VWF:CB/VWF:Ag) can be used in conjunction with VWF activity/VWF:Ag ratios to improve discrimination between type 2A/2B and 2M VWD.[77] There is no international agreement for a VWF:CB/VWF:Ag ratio cut-off to indicate type 2 VWD. VWF:CB/VWF:Ag ratio cut-offs used in studies vary between 0.4 and 0.8 with the specificity for type 2 VWD decreasing with increasing ratio.[77,84,85]

Recommendations

- Consider VWF:CB analysis on all new presentations with a laboratory phenotype of suspected type 2A/2B/2M that has been confirmed by repeat analysis (2B).
- Consider VWF:CB analysis as an additional test in an individual whose initial investigation is normal but in whom no other cause for a bleeding diathesis has been identified (2B).

Multimers

Traditional multimer analysis methods use sodium dodecyl sulphate (SDS) agarose gel electrophoresis followed by a visualisation technique such as alkaline phosphatase, chemiluminescence, autoradiography or infrared fluorescence.[86–91] In these methods, the concentration of agarose in the gel can be altered from the recommended intermediate resolution gel to focus on HMWM only (lower agarose concentrations) or abnormalities of the triplet structure using higher agarose concentrations.[87] A commercially available, semi-automated assay has also been validated

for use as a rapid screening test.[73,92] Improved reproducibility and detection of abnormalities are observed by quantifying multimers using densitometry.[93] Results of EQA suggest a consistent error rate of up to 15% with multimer assays; less interlaboratory variation is observed in normal or type 1 VWD samples.[94–96]

VWF multimer analysis is not only the definitive assay for differentiation between type 2A or 2B and 2M VWD but can also be useful in distinction between type 3 and very low type 1 VWD or in individuals with AVWS.[7,66,97–99] However, it must be recognised that results may be incongruent with some types of VWD. There have been reports of normal multimers in type 2B VWD[28,98] and some minor loss of HMWM linked to certain genetic variants have been reported in type 2M with the GPIb-binding type variants; normal multimer patterns are reported in type 2M with the CB-type variants[66,67,73,100]; ultra-large molecular weight multimers can be seen in type 1C VWD,[101] thrombotic thrombocytopenic purpura[73] and in some patients with type 2M VWD.[102]

Recommendations

- Consider multimer analysis in combination with densitometry on all new presentations with a laboratory phenotype of suspected type 2A/2B/2M that has been confirmed by repeat analysis (2B).
- Multimer analysis should only be performed in specialised haemostasis laboratories with experience of performing the analysis and interpretation of the results, and which participate in appropriate EQA (1C).

VWF binding to FVIII

Type 2N ('Normandy') VWD refers to an autosomal recessive disorder where there is abnormal binding of VWF to FVIII (VWF:FVIIIB)[103]; specific variants are present in the FVIII binding region of VWF (D′ D3 regions) causing low FVIII:C relative to VWF:Ag. Individuals present with a clinical and laboratory phenotype similar to mild haemophilia A but sometimes also including a low or near normal VWF:Ag. Differential diagnosis requires pedigree analysis and the identification of a reduced VWF:FVIIIB/VWF:Ag ratio (VWF:FVIIIB ratio)[104] typically ranging between <0.6 and 0.7.[105,106] Homozygotes and compound heterozygotes are likely to have a VWF:FVIIIB ratio of <0.3, whereas some heterozygous individuals may have a ratio of 0.3–0.7.[107,108] Overlapping borderline ratios of 0.6–0.7 can be obtained in otherwise normal blood donors identified with asymptomatic 2N variants.[109] Local reference ranges should be considered although access to 2N characterised samples is often limited. There is currently no international standard for VWF:FVIIIB, and no UK or European ISO17043-compliant EQA scheme, which adds to the uncertainty in the phenotypic assays. Genetic testing is therefore recommended in parallel with phenotypic assays unless results are clearly normal.

Recommendations

- Genetic testing for suspected type 2N VWD is recommended in individuals with a newly identified reduced FVIII:C/VWF:Ag ratio (1B).
- Consider a VWF:VIIIB assay in all newly identified individuals with suspected type 2N VWD, and those previously diagnosed as mild to moderate haemophilia A whose *F8* variant has not been identified or those with atypical bleeding and pedigree analysis (2C).

Ristocetin-induced platelet agglutination

Ristocetin-induced platelet agglutination (RIPA) is used as a marker of qualitative VWF binding to platelet glycoprotein Ibα.[110] Ristocetin at a high dose (1.25 g/L for light transmission aggregometry[35]) causes platelet aggregation in normal samples, but can sometimes be reduced in VWD. Aggregation with a lower dose of ristocetin (0.5 g/L for light transmission aggregometry[30,35]) is usually absent, but hyper-reactivity is associated with a type 2B VWD phenotype of spontaneous platelet aggregation and potential thrombocytopenia.[7,101] This hyperactivity is also seen in individuals with PT-VWD,[101] so mixing studies using normal donor plasma and normal platelets may be used in individuals presenting with low-dose ristocetin aggregation to differentiate between classical type 2B VWD and PT-VWD. Instructions for platelet mixing studies can be found in Table S2.[111]

Some patients with p.(Pro1266Gln) variants in *VWF* have hyper-reactivity to low-dose ristocetin but normal multimers and no thrombocytopenia.[112,113]

Genetic testing (for *VWF* and *GP1BA*) should be used for diagnosis of type 2B VWD or PT-VWD due to the limited certainty of the diagnostic evidence from phenotypic testing.[7]

Recommendations

- Genetic testing is recommended for individuals with suspected type 2B VWD or PT-VWD (1B).
- Consider performing a RIPA in individuals with suspected type 2B or PT-VWD using two concentrations of ristocetin (2B).
- The presence of aggregation with low-dose ristocetin should be confirmed by repeat testing (1B).
- Consider donor mixing studies in patients with aggregation with low-dose ristocetin (2B).

VWF pro-peptide assay

On secretion into the circulation, the VWF pro-peptide (VWFpp) dissociates from mature VWF protein and circulates with a half-life of 2–3 h.[114] Type 1 and type 3 VWD cause a 1:1 reduction of VWFpp compared to VWF:Ag and

detection of low levels of VWFpp may differentiate severe type 1 VWD from type 3 VWD.

Ratios of VWFpp/VWF:Ag of 0.6–1.6 are seen in normal individuals and 1.5–2.7 (interquartile range) in individuals with type 1 VWD,[115] whereas much higher ratios (8.4–13.5) have been reported in individuals with the p.(Arg1205His; Vicenza) variant, inversely proportional to the increased clearance of VWF in response to trials of desmopressin (DDAVP).[115–117] Ratios of VWFpp/VWF:Ag are also moderately increased in patients with type 2 VWD (2.0–2.8) and in those with AVWS (4.0–10.5), except those with AVWS secondary to essential thrombocythaemia (1.2–1.5).[115]

Commercial ELISAs to measure VWFpp are not readily available. Recent guidelines[7] suggest when a DDAVP trial is performed, the measurement of VWF antigen and activity, rather than measurement of VWFpp, is performed in individuals with suspected enhanced clearance, and this should be followed up by genetic testing.

Recommendations

Genetic testing is suggested for individuals with reduced VWF half-life to desmopressin rather than performing a VWFpp assay (1B).

MEASUREMENT OF INHIBITORS IN HERITABLE VWD AND AVWS

Anti-VWF alloantibodies develop in 5%–10% of individuals with type 3 VWD[118] and can be associated with anaphylaxis.[119] Risk factors for inhibitor development include variants resulting in null alleles, typically deletions,[120] and a family history of inhibitors.[121] The antibodies are immunoglobulin G type.[119]

There is no internationally agreed laboratory assay for diagnosis of VWF inhibitors. Mixing studies are often negative, although there is variation in sensitivity depending on which assay is chosen to measure residual VWF activity. VWF:CB testing may be the most sensitive: in the 3WINTER IPS study, 13 individuals with inhibitors were detected by this method; 10/13 were also detected by VWF:GPIbM and only 6/13 by VWF:Ag assays.[122]

Anti-VWF antibodies do not demonstrate time or temperature dependence, and the incubation of the mixed samples can be performed at 37°C for between 15 min and 2 h,[118] with results reported in Bethesda units. Testing should be performed in an experienced laboratory familiar with the assay. Results may be negative if the anti-VWF antibodies are directed against non-functional epitopes.[118]

The pathophysiology of AVWS is complex and not necessarily related to anti-VWF autoantibodies.[123] Additionally, in those cases where there is an anti-VWF autoantibody, mixing studies rarely identify an inhibitor. Antibodies that bind to VWF and accelerate VWF clearance without affecting VWF activity cannot be detected by this method; anti-VWF ELISAs are an option but are not commercially available and not standardised. Therefore the absence of a demonstrable inhibitor does not exclude a diagnosis of AVWS.

Recommendations

- An initial 50:50 mix with normal plasma followed by measurement of VWF by an activity assay is suggested to detect an anti-VWF inhibitor (2B).
- Further dilutions of patient plasma in buffer may be used if an inhibitor titre is required (2B).
- Genetic testing can be considered in the differential diagnosis between AVWS and heritable VWD (2C).

USE OF GENETIC OR GENOMIC TESTING

Even though a VWD diagnosis is based on clinical and laboratory phenotype, genetic information can complement or confirm a diagnosis, and support family studies, particularly where the pattern of inheritance is unclear and/or variable. Genetic diagnosis may indicate an alternative diagnosis when there are conflicting phenotypes and clinical symptoms.[124] However, the pattern of inheritance remains elusive for many published *VWF* variants, with incomplete penetrance and variable expressivity detected within and between families.[2] Moreover, insufficient data from additional affected and unaffected family members can hinder the determination of the pattern of inheritance of novel variants.

Type 3 and type 2N VWD exhibit autosomal recessive inheritance, whereas type 1 and other type 2 VWD typically exhibit autosomal dominant inheritance.[125] Although mosaicism in VWD is uncommon, parents of affected offspring have been reported as unaffected and not found to carry the familial variant (gonadal mosaicism),[126] or unaffected but carrying the familial variant at a reduced percentage in blood and oral mucosal cells (somatic mosaicism).[127]

The increased data from high-throughput molecular technologies have led to a vast number of variants within *VWF* being discovered, whose clinical significance is not yet fully understood. This, coupled with incomplete availability of clinical data, may make defining a variant as pathogenic or benign extremely difficult. Laboratories are encouraged to report all new variants and associated clinical and laboratory phenotypes to international databases.

Introduction to the gene and pseudogene

The VWF gene (*VWF*) is located on the short arm of chromosome 12 (12p13.3).[128,129] It consists of 178 kb of genomic DNA distributed across 52 exons, with exon 28 being the largest, spanning 1.4 kb.[130] The gene produces a 9 kb mRNA which encodes for a 2813 amino acid (aa) precursor protein designated prepro-VWF.[131,132] It consists of a 22-aa signal (pre) peptide, a 741-aa pro-peptide and a 2050-aa mature VWF subunit, with a repeating domain structure (Figure 2). *VWF* has a partial pseudogene (*VWFP*) which is located on the long arm of chromosome 22 (22q11.22), with

a 97% homologous region corresponding to exons 23–34 of *VWF*.[133] Along with a large number of variants classified as benign or of unknown significance, these features complicate the genetic analysis and interpretation of detected variants within individuals with reduced VWF.

Diagnostic strategy

With index cases, laboratory assays for VWD should usually be performed twice before proceeding to *VWF* genetics, although clinical exceptions may arise. Exon 28 encodes the functional A1 and A2 domains and variants within this region have been found in all subtypes of type 2 VWD[1,134,135]; targeted analysis of this region can be informative in these individuals.[2] However, limitations must be considered where a variant has not been detected in exon 28, as the possibility of variants elsewhere within *VWF* may need to be addressed.

A flow chart for the laboratory investigation of suspected VWD and/or AVWS can be seen in Figure 1. It should, however, be noted that there are always exceptions to these indications, due to the heterogeneity of VWD.

As the capacity of genetic data increases in laboratories, so does the occurrence of incidental findings, therefore caution should be shown when assessing a *VWF* variant for pathogenicity. Some common single-nucleotide variants (SNVs) within *VWF*[61] have been found at high frequency in the general population and are risk factors for reducing VWF activity in some assays, but are not associated with a bleeding phenotype when inherited in isolation.[136–138] Therefore, if an individual has inherited a *VWF* variant that is classified as benign, likely benign, or a variant of uncertain significance, then they should be investigated for other haemostatic defects.

Methodology and interpretation

In England, the NHS National Genomic Test Directory indicates that *VWF* genetic testing may be requested by Consultant Haematologists or Clinical Geneticists by requesting either R121 (single gene analysis) or R90 (Bleeding and Platelet Disorders panel).[139] Phenotypic data should be provided with each referral to aid in analysis of results.

When choosing an analytical technique, molecular laboratories must be aware of the sensitivity and specificity of the approach used and the turnaround time for producing an interpretative report. There are a range of methodologies available for the genetic investigation of *VWF*, and the availability of different platforms will vary depending on the resources available to the genetic testing laboratory. In the United Kingdom and Ireland, the analysis of the essential regions of *VWF* is usually either by next-generation sequencing (NGS) or by direct DNA sequencing.

The analysis of the *VWF* gene should include the investigation of the coding regions (52 exons), flanking regions including the splice site boundaries, and the 5′ and 3′ untranslated regions. Special consideration must be made to the presence of the *VWFP* pseudogene when designing assays for analysis of *VWF*. All results of genetic testing should be confirmed by independent testing of the original DNA sample. This may be accomplished either through a repeat of the original assay or by using a different methodology, or by testing a second sample. Any limitations in the assay or technology used should be acknowledged in the interpretative report.

NEXT-GENERATION SEQUENCING

NGS allows the simultaneous study of many genes and has been used to study large panels of genes associated with

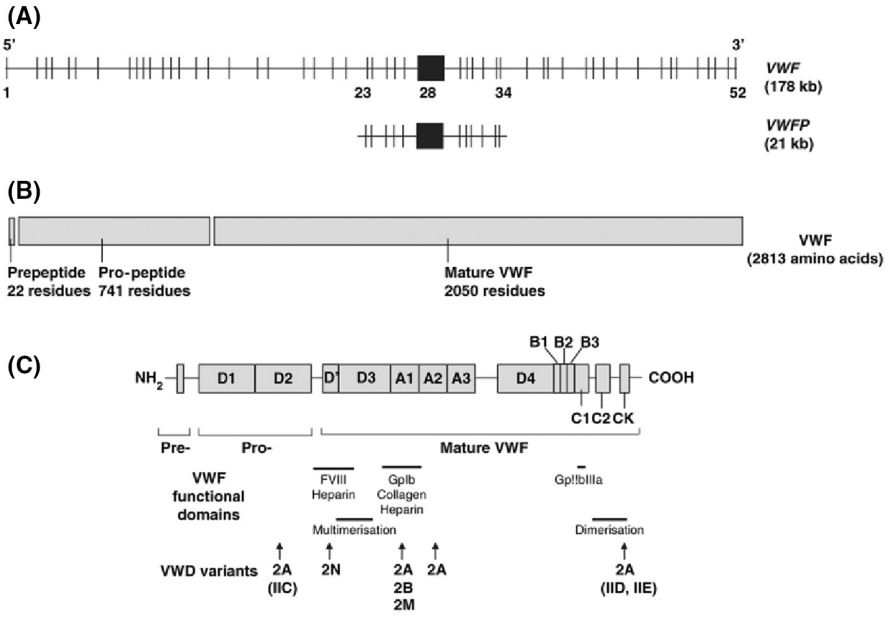

FIGURE 2 Schematic layout of the VWF domains. Not to scale. (A) The structure of human *VWF* and *VWFP*; (B) prepro-VWF; (C) functional domains of VWF and locations of common variants associated with type 2 VWD.[2]

bleeding and platelet disorders.[140–143] Bioinformatics pipelines allow for genes in the panel to be analysed individually, therefore for an individual referred for *VWF* genetic testing, the data for *VWF* can be filtered from a larger panel/exome and analysed. NGS analysis can identify SNVs, small deletions, insertions/deletions and large copy number variants (CNVs). Different NGS platforms are available from various manufacturers.

When analysing *VWF* by NGS, it may be possible that some sequence reads map to the *VWF* pseudogene, and therefore variants in *VWFP* may be incorrectly filtered in for data analysis. If this is the case for the NGS technology employed, variants detected in exons 23 to 34 of *VWF* (the region that is homologous to the *VWFP*), should be confirmed, ideally using a different analytical technique, to ensure the specificity of the variant to *VWF*. Some NGS technologies may not be able to map sequence reads to specific areas of the gene, such as exon 26 in *VWF*. Such regions should therefore be analysed by Sanger sequencing.

DNA SEQUENCING BY SANGER SEQUENCING

Streamlined DNA sequencing methods by Sanger sequencing, including automated or semi-automated procedures, can generate full sequence data for *VWF* within a rapid timescale. There is no standard primer set recommended for the amplification of the essential regions of *VWF*, but it is important to consider the following when designing primers, or using those previously published:

- Primer sequences should be regularly checked for underlying SNVs to avoid unspecific allele amplification.
- Tailed primers (for example with M13 sequence) are recommended to facilitate efficient downstream sequencing protocols.
- Care must be taken to ensure primer specificity to *VWF* in exons 23–34, where there is high homology with *VWFP*. Primer sequences must be designed to be specific to *VWF*. This can be achieved by locating the primers with the 3′ most base in the location of a base mismatch between *VWF* and *VWFP*. By including an additional mismatch in the primer sequence at 2 or 3 bases from the 3′ end, this will increase specificity of the primer for *VWF*.

CNV ANALYSIS

CNV analysis to detect the presence of large deletions or duplications of *VWF*, from a single exon to the full gene, may be performed using techniques such as NGS, multiplex ligation-dependent probe amplification (MLPA) or droplet digital polymerase chain reaction. When using NGS, the read depth should be sufficient to allow for accurate detection of CNVs, as determined by in-house validation of the assay. Commercial MLPA kits are available for analysis of *VWF*. Any CNVs identified should be confirmed by repeat analysis of the analytical sample.

CLASSIFICATION AND DESCRIPTION OF VARIANTS

Interpretation of *VWF* variants must be performed by specialist clinical scientists with the skills to research and interpret the significance of any variants detected, due to the complexity of the gene. All variants in *VWF* should be classified according to ACMG guidelines[3,4] and ACGS best practice guidelines for variant interpretation.[5]

All *VWF* variants should be described using Human Genome Variation Society (HGVS) nomenclature[144] to ensure international standardisation of variant naming. The reference sequence and version number used for analysis should be included in the report to ensure that historical variants can be identified and investigated if there are future changes to the genomic reference sequence. The reference sequence used for *VWF* is currently NM_000552.[145]

VWF variant databases

Databases such as the EAHAD *VWF* variant database (https://dbs.eahad.org), Human Gene Mutation Database (https://www.hgmd.cf.ac.uk) and the ClinVar variant database (https://www.ncbi.nlm.nih.gov/clinvar) can be interrogated to see whether a variant has previously been identified and classified. They should be used with caution as variants may have been submitted prior to the change in definition of VWD based on VWF activity <30 IU/dL[1,7]: some previously classified variants may now not be recognised as being associated with VWD. In addition, some variants may have been described as pathogenic prior to the introduction of the ACMG guidelines for variant classification.[3] Table S3 shows VWF variants with a MAF >0.0001 that are currently classified as having conflicting data on pathogenicity; this list is not exhaustive and variants are constantly being reviewed for their association with VWD.

Crystal structures of the VWF domains from the Research Collaboratory for Structural Bioinformatics Protein Data Bank[146] can be used to visualise the location of the amino acid within the tertiary protein structure and assess the likely pathogenicity of a novel variant.

Reporting of results

Interpretative VWF laboratory reports should be clear, concise and follow the ACGS[5] and European Society of Human Genetics[124] best practice guidelines. Where laboratory and clinical data are key to classification of a specific variant, a multidisciplinary team involving clinicians

and scientists should form the part of the reporting process; family studies may assist in this classification of variants and to determine penetrance. All individuals with a confirmed diagnosis should be consented for inclusion on the National Haemophilia Database and offered a bleeding disorder card.

Recommendations

- Genetic analysis (R121, *VWF*) may be considered in individuals with VWF activity (by any activity assay) or antigen <30 IU/dL that has been found on two occasions (2C).
- Genetic analysis (R90) may be considered in individuals with VWF levels between 30 and 50 IU/dL if no other causes for a bleeding phenotype have been identified (2B).
- Clinicians should provide clinical and laboratory phenotypic details with each referral including results of relevant assays (2B).
- Informed consent must be obtained prior to referral of an individual for genetic testing, including providing information regarding potential incidental findings (1B).
- Techniques used for genetic analysis must have high sensitivity and specificity for the detection of SNVs and CNVs in *VWF* (1B).
- Interpretive reports should follow ACMG and ACMS guidelines and variants described according to HGVS nomenclature (1B).

LABORATORY TESTING TO GUIDE MANAGEMENT

Measuring response to desmopressin (DDAVP)

The clinical use of DDAVP in the treatment of VWD is well reviewed and detailed in the previous UKHCDO guideline.[1] The response to DDAVP in temporarily raising FVIII and VWF levels varies widely such that a trial of desmopressin is recommended in individuals with type 1, type 2A, type 2M and type 2N VWD prior to its use. For individuals with VWD, the monitoring of FVIII:C (by OSCA or CSA), VWF activity and VWF:Ag should be measured at all timepoints.[1]

There are little data regarding the monitoring of response to DDAVP using VWF:GPIbR or VWF:GPIbM assays. However, the assays have been well described in measuring VWF activity,[59] and the ability to measure VWF activity rapidly and accurately supports their use as functional measurement in monitoring response to DDAVP.

Only one VWF activity assay (VWF:RCo or VWF:GPIbR or VWF:GPIbM) needs to be assayed to monitor response to DDAVP.

Measuring response to VWF concentrates

VWF concentrates may be plasma derived, with FVIII in varying concentrations and with or without normal distribution of HMWMs, or recombinant containing no FVIII. The potencies of all VWF concentrates are currently assigned using the VWF:RCo assay.[147]

Treatment is monitored by the measurement of FVIII:C (by OSCA or CSA) and VWF activity. There is a relative paucity of data on the use of VWF:GPIbR or VWF:GPIbM assays in monitoring of VWF concentrate treatment.[148,149] Field studies should be performed and published on VWF concentrates, but until more conclusive data are available, laboratories should consider performing local verification using either VWF:GPIbR or VWF:GPIbM if these assays are to be used. However, the assays have been well described in measuring VWF activity,[59] and the ability to measure VWF activity rapidly and accurately supports their use as functional measurement in monitoring VWF concentrate infusions.

Assays calibrated with plasma standards, traceable to WHO International Standard for Plasma FVIII and VWF should be used for monitoring VWF concentrate infusions, unless there is specific evidence to the contrary.

Only one verified VWF activity assay (VWF:RCo or VWF:GPIbR or VWF:GPIbM) needs to be performed to measure response to VWF concentrates.

Measuring VWF during pregnancy

Testing for diagnostic purposes should be avoided during pregnancy, and therefore pregnancy-specific reference ranges are not required. However, individuals with known VWD may require monitoring during pregnancy. FVIII:C (by OSCA or CSA), VWF activity and VWF:Ag should be measured in these situations.

Only one VWF activity assay (VWF:RCo or VWF:GPIbR or VWF:GPIbM) needs to performed to monitor VWF levels in pregnancy.

Measuring VWF in neonates/infants

The diagnosis of VWD in neonates and infants is complicated by preanalytical variation due to the difficulty in obtaining a non-activated and uncontaminated venous or cord sample and the prolonged physiological increase in VWF following delivery. However, when testing is clinically necessary, a severe deficiency or type 2 disease should be apparent. In these cases, a minimum panel including a clotting screen and FVIII:C (by OSCA or CSA), VWF activity (by VWF:RCo or VWF:GPIbR or VWF:GPIbM) and VWF:Ag should be performed, and results compared to age-specific reference ranges. Generating local age-specific reference

ranges is likely to be unfeasible for most laboratories, so published ranges can be considered if based upon similar analyser/reagent combinations; otherwise, referral to a specialist centre that can report age-specific reference ranges may be considered.[11,150] In one multicentre study, VWF:Ab and VWF:Ag levels mirrored those in adults by 6 months of age, but VWF:GPIbR levels were still lower than adult levels up to 1 year of age.[151] Genetic testing may be useful to aid interpretation.

Recommendations

- For individuals with VWD, FVIII:C (by OSCA or CSA), VWF activity (by VWF:RCo or VWF:GPIbR or VWF:GPIbM) and VWF:Ag should be measured to assess response to DDAVP and/or to monitor levels in pregnancy (1B).
- In the absence of field studies, laboratories may consider performing local verification of VWF:GPIbR or VWF:GPIbM assays if they are to be used to monitor response to VWF concentrates (2D).
- For individuals with VWD, FVIII:C (by OSCA or CSA) and VWF activity (by VWF:RCo or VWF:GPIbR or VWF:GPIbM) should be measured to assess response to VWF concentrates (1B).
- When urgently required in neonates or infants to exclude a diagnosis of VWD, FVIII:C (by OSCA or CSA), VWF activity (by VWF:RCo or VWF:GPIbR or VWF:GPIbM) and VWF:Ag should be measured (1B).

AUTHOR CONTRIBUTIONS

All authors contributed equally to the writing of the text and review of each draft.

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REVIEW PROCESS

Members of the writing group will inform the writing group Chair if any new evidence becomes available that would alter the strength of the recommendations made in this document or render it obsolete. The document will be reviewed regularly by the relevant Task Force and the literature search will be rerun every 3 years to search systematically for any new evidence that may have been missed. The document will be archived and removed from the BSH current guidelines website if it becomes obsolete. If new recommendations are made, an addendum will be published on the BSH guidelines website (https://b-s-h.org.uk/guidelines).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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