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Testing for von Willebrand Disease

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SYNOPSIS AND RELEVANCE

Von Willebrand disease (VWD) is the most common inherited bleeding disorder and has an autosomal inheritance pattern.[1] An acquired form can be seen in patients secondary to a wide variety of illnesses including autoimmune disease, malignancies, cardiac disease, and others. VWD results from either a quantitative deficiency or functional defect (qualitative) in von Willebrand Factor (VWF), a plasma glycoprotein required for proper hemostasis by mediating adhesion of platelets at sites of blood vessel injury.[2-7] The clinical findings include excessive bleeding from small cuts, bleeding from mucous membranes, and easy bruising, which may be spontaneous or following mild trauma. Commonly, patients with VWD experience mild to moderate symptoms resulting from the downstream reduced platelet adhesion, whereas other individuals experience no symptoms at all. VWD is classified into the subtypes 1, 2, and 3, with Type 2 subdivided into type 2A, 2B, 2M, and 2N. Additionally, platelet mutations result in a VWD appearing disease termed Platelet Type Von Willebrand disease (PT-VWD). Diagnosis of several of these subtypes may be challenging but is important because treatment depends on the proper subtype classification. This module serves to illustrate the relevant tests and appropriate algorithm to assist in diagnosis and proper subtype classification of VWD.

OBJECTIVES

1. Identify the initial tests required when evaluating a patient with a history of an inherited bleeding disorder.
2. Understand the importance of the VWF activity/VWF:Ag (antigen) ratio in the diagnosis of VWD.
3. Know when to order VWF multimeric analysis in the diagnosis of less common VWD subtypes.

BACKGROUND

VWF is a multimeric glycoprotein synthesized by endothelial cells and megakaryocytes and circulates in the blood as a chaperone protein for Factor VIII.[4] VWF facilitates the adherence of platelets to subendothelial collagen at sites of blood vessel injury. VWF interacts with the platelet Glycoprotein Ib (GP Ib) receptor and stabilizes factor VIII in the bloodstream. Multimeric VWF is comprised of high, intermediate, and low molecular weight (HMW, IMW, and LMW) multimers.

Evaluation for a suspected inherited bleeding disorder involves a thorough documentation of the patient's bleeding history and family history.[2,5,7,8] If the history is suspicious for an inherited bleeding disorder, a platelet count, prothrombin time (PT) test, partial thromboplastin time (aPTT), and fibrinogen test should follow as the initial screening panel.

Most commonly, patients with von Willebrand Disease present with a normal platelet count and coagulation profile.[2] Certain subtypes of von Willebrand Disease are associated with decreased platelet count and the aPTT may be prolonged if FVIII is low.[4,9]

When VWD is suspected based on clinical presentation, guidelines recommend ordering the following quantitative tests: VWF:Ag, VWF activity (several different activity tests are available), and a factor VIII level in order to diagnose VWD and determine the VWD subtype. As VWF is an acute phase reactant, testing should be done when other clinical conditions may confound the test results. The VWF:Ag determines the total protein VWF level.[4] Von Willebrand activity assesses the functional activity of VWF (usually platelet-binding activity). The historic Ristocetin Cofactor activity assay (VWF:RCo) determines the agglutination of platelets in the presence of the antibiotic ristocetin, which activates VWF and allows it to interact with platelet glycoprotein 1b (GP1b) receptor. Newer assays replacing VWF:RCo, such as VWF:GPIbM, VWF:Ab, and GP1bR, are available to assess VWF activity and have the advantage of being unaffected by common ristocetin binding polymorphisms.[10] In order to diagnose Type 1 VWD, the most common subtype of VWD, the aforementioned testing is often sufficient. The less common subtypes, however, may require additional testing such as VWF multimeric analysis by gel electrophoresis.[4] **Appendix II** provides a sample algorithm for VWD testing.

Type 1 VWD involves quantitative deficiencies in VWF. Type 3 VWD involves the complete loss of von Willebrand factor. In contrast, Type 2 VWD results from qualitative defects in VWF.[2,4] Specific subtypes reflecting abnormal VWF function result from:

a) Missing large VWF multimers: 2A, 2B, PT-VWD
b) Loss of function mutations in platelet binding or collagen binding domains of VWF: 2M
c) Loss of function mutations in factor VIII binding domain of VWF: 2N.

If there is a proportionate (also known as “concordant”) decrease between VWF antigen and activity, with an activity to antigen ratio between 0.7 and 1.0, a quantitative subtype of VWD should be considered (Type 1). Alternatively, if there is a disproportionate decrease (also known as “discordant”) [i]between activity to antigen, with a ratio <0.7, types 2A, 2B, and PT-VWD should be considered.

In Type 2M VWD, there is a defect in the platelet binding or collagen binding domain of VWF, with multimeric subunits present. In Type 2M VWD, there is a defect in the platelet binding or collagen binding domain of VWF, with multimeric subunits present. Additional testing with VW.CB/Ag is helpful to establish this diagnosis (see Appendix II).[11]

In the rare Type 2N VWD, a mutation in the factor VIII binding domain of VWF results in a decreased Factor VIII level. Genetic analysis or VWF:FVIIIB or enzyme-linked immunosorbent assay (ELISA) are available to establish the diagnosis of VWD type 2N.[4,10]

Type 2B and PT-VWD are the result of gain-of-function mutations in VWF or platelet GP1b receptor, respectively.[9] These mutations cause increased interactions between VWF and platelets, and these complexes are continuously cleared from circulation, leading to the loss of HMW multimers of vWF and decreased platelets in circulation.[4] The typical clinical presentation of patients with Type 2B vWD or PT-VWD is mild thrombocytopenia with the absence of HMW VWF multimers on gel electrophoresis studies.

Therefore, in order to differentiate between Type 2B and PT-VWD, one must determine whether there is a platelet or VWF defect.[4,12] Traditional low-dose ristocetin-induced platelet aggregation studies (LD-RIPA) studies are used to assess for the presence of VWF type 2B but do not differentiate the two types because these assays utilize both patient platelets and VWF. Gene sequencing may be required to distinguish between these two subtypes when both are suspected.

The subtyping of VWD is crucial to treatment decisions, as use of desmopressin (DDAVP) is controversial for treatment of VWD type 2B, less effective in type 2 overall, and is ineffective in type 3 disease.[13]

Appendix III provides an overview of diagnostic laboratory criteria for each type of VWD.

INSIGHTS

Initial testing to determine diagnosis of Von Willebrand Disease should be limited to complete blood count (CBC) with platelet count to rule out quantitative platelet disorders, followed by VWF:Ag and VWF activity, and factor VIII level.[2-4]

1. VWD multimer analysis by gel electrophoresis should not initially be ordered to determine if a patient has VWD.[4]
2. VWD multimer analysis by gel electrophoresis is only necessary to assist in diagnosis if a qualitative subtype of VWD (inherited or acquired) is suspected, in which the ratio reveals a discordant pattern.[2-4]

INTERVENTIONS

1. Emphasize consensus guidelines regarding the appropriate initial testing required when working up a patient with suspected VWD.
2. Review test ordering practices from laboratory information systems about what initial VWD specific tests are ordered when evaluating a patient with suspected VWD.
3. Review test ordering patterns from laboratory information systems about how often VWD multimer analysis by gel electrophoresis is appropriately ordered when evaluating patient for VWD .
4. Add comments in the laboratory information system to inform clinicians about the appropriate use of VWF multimer analysis by gel electrophoresis. Consider a stop/alert in the laboratory information system to reduce the numbers of inappropriately ordered VWF multimeric analysis.
5. Review the appropriateness of VWD test ordering patterns on a regular basis to assess the impact of the interventions (see Impact Analysis below).
6. Develop an order set consistent with guidelines and algorithms required for initial diagnosis and proper classification of VWD.

INTERVENTION ANALYSIS

The intervention analysis calculation is found in **Appendix I**.

1. Determine the number of VWF:Ag and activity (such as VWF:RCo) tests ordered in a given time period, eg, three months, annually. (A1)
2. Determine the number of VWF multimer analysis by gel electrophoresis ordered initially with VWF:Ag and activity over the same period of time above. (A2)
3. Assess the impact volume by calculating the percentage difference (A3) in the total number of patients who had test orders for VWF:Ag and activity (A1) and those who had VWD multimeric analysis was ordered unnecessarily. (A2)
4. Following implementation of interventions (education of practitioners, order entry modifications) determine number of VWD multimeric analysis ordered over the same time interval analyzed pre-intervention and the percent difference. (B1, B2, B3)
5. Calculate the percent reduction in VWD multimer analysis ordered following implementation of interventions described above.(C3)

APPENDICES

APPENDIX I. MEASURING THE INTERVENTION ANALYSIS

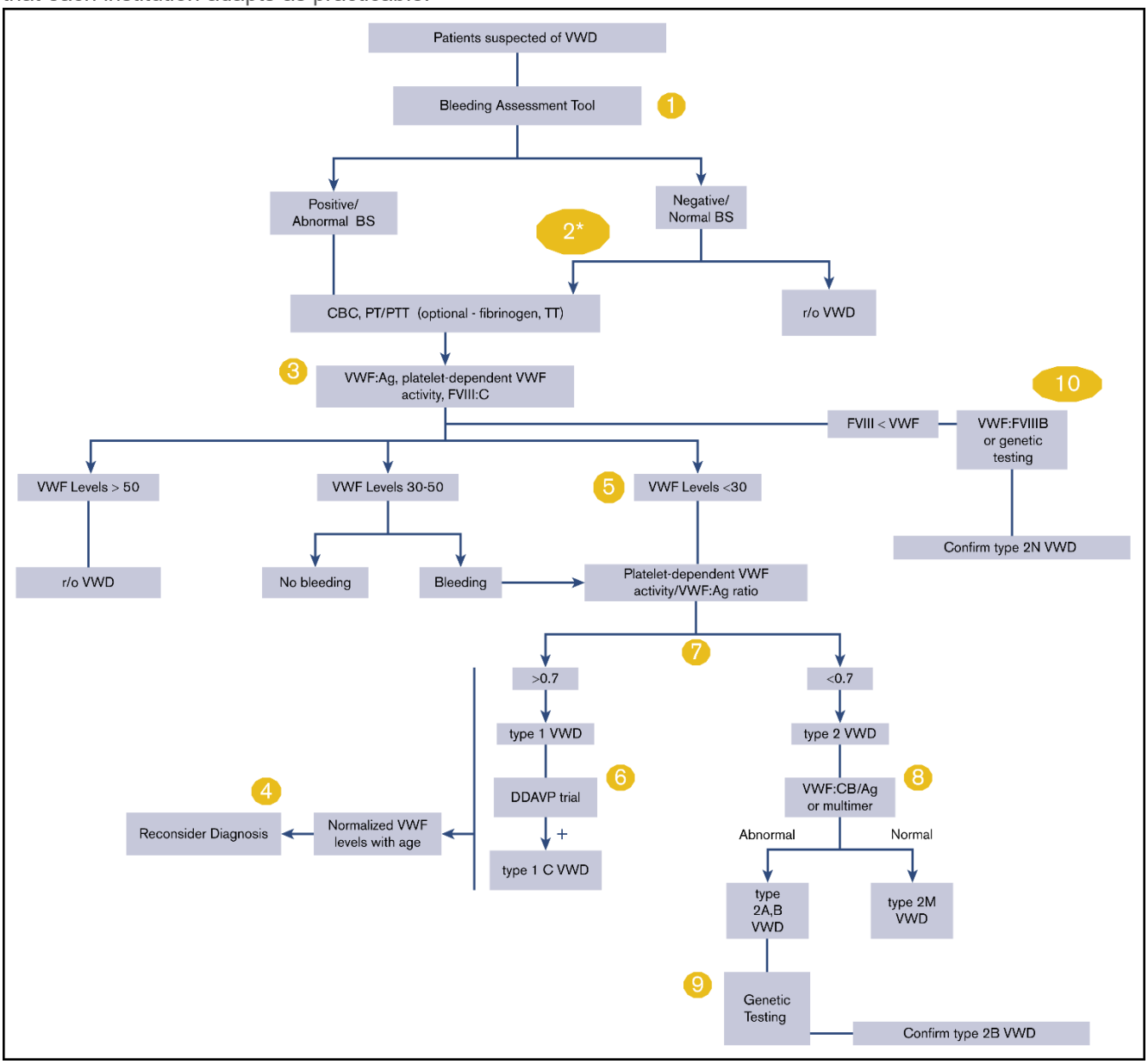
This activity is done most easily using the same time period for each measurement, eg, 3 months, or one year.

Test Volume	Pre Intervention	Post Intervention	Pre-Post Intervention Change
Number of VWF:AG + activity ordered in a given time period	A1	B1	A1- B1 = C1
Number of VWF multimer analysis by gel electrophoresis ordered initially with VWF:Ag and activity	A2	B2	A2 – B2 = C2
Number of inappropriately ordered VWF multimer analyses (initial order)	(A1-A2)/A1 x 100% = A3%	(B1-B2)/B1 x 100% = B3%	A3% - B3 %= C3%

APPENDIX II.

AN OVERALL ALGORITHM ADDRESSING THE DIAGNOSIS OF VWD [11,a,b]

This flowchart was published in *Blood Advances,* and incorporates testing guidelines. We recommend that each institution adapts as practicable.



Abbeviations: BS, bleeding score; CBC, complete blood count; DDAVP, desmopressin; FVIII, factor FVIII; FVIII:C, FVIII coagulant activity; PT, prothrombin time; PTT, partial thromboplastin time; r/o, rule out; TT, thrombin time; VWF:CB/Ag, ratio of VWF collagen binding to antigen; VWF:FVIIIB, VWF FVIII binding.PT, prothrombin Time; PTT, partial thromboplastin time; VWD, Von Willebrand disease.

[11,a] This figure was republished with permission from *Blood Adv.* 2021; 5 (1): 280–300. ASH ISTH NHF WFH 2021 guidelines on the diagnosis of von Willebrand disease. doi: https://doi.org/10.1182/bloodadvances.2020003265. American Society of Hematology.

[11,b] The numbers in the yellow circles correspond to guideline questions in ASH ISTH NHF WFH 2021 Guidelines on the Diagnosis of von Willebrand Disease published in *Blood Advances* . VWF levels refer to VWF antigen (VWF:Ag) and/or platelet-dependent VWF activity. The algorithm says VWF level 30 to 50 for simplicity; this refers to VWF levels of 0.30 to 0.50 IU/mL, with the caveat that the lower limit of the normal range as determined by the local

laboratory should be used if it is <0.50 IU/mL. *Men and children, referred to a hematologist and/or first-degree relative affected with VWD.

APPENDIX III.
DIAGNOSTIC LABORATORY CRITERIA FOR EACH TYPE OF VON WILLEBRAND DISEASE[a]

Type	VWD Type 1	VWD Type 2	VWD type 3
Subtype	Classic 1C	2A 2B 2M 2N	
Frequency	Common (70% of cases)	Uncommon (25% of cases)	Rare (5% of cases)
Pathophysiology	Quantitative deficiency	Qualitative defects in VWF	Near complete quantitative deficiency
Inheritance	AD	AD (2A, 2B, 2M), AR (2N)	AR
Clinical phenotype	Mild to moderate	2A, 2B - Moderate to severe 2M - Severe 2N – Hemophilia-like bleeding	Severe and hemophilia-like
Ratio of VWF activity to VWF antigen	Normal (about 1)	< 0.7	Markedly low or undetectable VWF activity and antigen
Factor VIII levels	Normal or mildly low	Normal or mildly low (2A, 2B, 2M) Moderately low relative to VWF antigen (2N)	Very low
VWF multimer analysis	All present but at low level	2A: Absence of high and intermediate molecular weight multimers 2B: Absence of high molecular weight multimers 2M, 2N: Normal multimer pattern	Minimal or complete absence
Special Testing	None	Genetic testing RIPA: increased 2B and platelet type, decreased 2M 2N: decreased binding of VWF to F8 and prolonged aPTT	Genetic testing

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; aPTT, activated partial thromboplastin time; F8, Factor 8; RIPA, Ristocetin-induced platelet aggregation; VWF, von Willebrand factor.

[a,14]Appendix III is adapted with permission from Kaur V. Von Willebrand disease: A guide for the internist. *Cleve Clin J Med*. 2024;91(2):119-127. doi:10.3949/ccjm.91a.22033

QUESTIONS AND ANSWERS

QUESTION 1
QUESTION 1 OBJECTIVE
Identify the initial tests required when evaluating a patient with a history of an inherited bleeding disorder.

A young patient presents to the clinic with a family and personal history of abnormal and prolonged platelet-type/primary hemostatic bleeding. The initial test panel should include:

A. Platelet count, VWF:Ag and VWF activity
B. Platelet count, PT, aPTT, fibrinogen
C. VWD Multimer Analysis
D. Factor VIII, VWF:Ag and VWF activity

The correct answer is B. Basic coagulation studies (PT, aPTT, fibrinogen) and platelet count are necessary and the first step when working up any bleeding disorder in order to determine whether the defect lies in the coagulation factors, platelets, both, or none.
A is incorrect. VWF:Ag and VWF activity are ordered when specifically suspecting vWD.
C is incorrect. This test should be ordered when determining von Willebrand Disease Subtype, specifically Type 2 VWD.
D is incorrect. This panel of tests should be ordered during the initial evaluation of a patient specifically when VWD is suspected.

QUESTION 2
QUESTION 2 OBJECTIVE
Understand the importance of the VWF activity to antigen ratio in the diagnosis of VWD.

A patient being worked up for VWD has a VWF activity 24 IU/dL (normal 50-200), and the VWF antigen 30 IU/dL. What is the best interpretation of these results?
A. Quantitative subtype of VWD, concordant ratio
B. Quantitative subtype of VWD, discordant ratio
C. Qualitative subtype of VWD, concordant ratio
D. Qualitative subtype of VWD, discordant ratio

The correct answer is A. Ratio is 0.8 indicating a quantitative subtypes of VWD are associated with a concordant VWF activity to antigen ratio, indicating a proportionate decrease between the vWF antigen level and its associated activity.
B is incorrect. A ratio of 0.7-1.0 indicates a concordant ratio (not discordant), suggesting a quantitative subtype of VWD.
C is incorrect. A ratio of 0.7-1.0 indicates a concordant ratio, suggesting a quantitative (not qualitative) subtype of VWD.
D is incorrect. A ratio of 0.7-1.0 indicates a concordant ratio (not discordant), suggesting a quantitative (not qualitative) subtype of VWD.

QUESTION 3
QUESTION 3 OBJECTIVE
Know when to order VWF multimeric analysis in the diagnosis of VWD.

A patient being worked up for vWD has a VWF activity to antigen ratio of 0.6. What testing, if any, should be ordered as a follow-up to this result?
A. None, this testing is sufficient to determine patient has a quantitative subtype of vWD.
B. Targeted Genetic Mutation Analysis
C. VWD Multimer Analysis by Gel Electrophoresis
D. Factor VIII level

The correct answer is C. VWD Multimer Analysis by Gel Electrophoresis is necessary to determine the diagnosis for qualitative subtypes of VWD, namely type 2A, 2B, 2M, and PT-VWD.
A is incorrect. A ratio of 0.6 indicates a discordant ratio, suggesting a qualitative subtype of VWD, either type 2 or PT-VWD. VWD multimer analysis is necessary to determine the presence or absence of specific multimeric subunits of the entire vWF protein. This in turn, helps determine the qualitative subtype (Type 2A, 2B, 2M or PT-VWD) to be diagnosed.
B is incorrect. Targeted genetic mutation analysis is required when one needs to differentiate between Type 2B VWD and PT-VWD.
D is incorrect. A factor level is required in the initial evaluation of VWD. This test helps to exclude VWD Type 2N.

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