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Pearls of Laboratory Medicine
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TITLE: von Willebrand Disease

PRESENTER: Kristi J. Smock, MD

Slide 1:
Hello, my name is Kristi Smock. I am an Associate Professor of Pathology at the University of Utah Department of Pathology and a Medical Director for the Hemostasis/Thrombosis Laboratory at ARUP Laboratories. Welcome to this Pearl of Laboratory Medicine on "**von Willebrand Disease**."

Slide 2:
von Willebrand factor (vWF) is a multimeric protein that mediates adhesion of platelets at sites of vascular injury by interacting with exposed collagen and the platelet glycoprotein 1b (GP1b) receptor on the platelet surface. Circulating vWF in the bloodstream does not interact strongly with platelets. Collagen is not exposed to the bloodstream in normal conditions. When there is vascular injury, vWF becomes tethered to exposed collagen via its collagen-binding domains, and the shear forces of flowing blood induce a conformational change that expose the vWF platelet-binding domains, helping platelets adhere to the site of injury. The high-molecular-weight (HMW) forms of vWF are composed of more vWF subunits and have greater ability to bind to ligands such as collagen and platelets than the smaller low-molecular-weight (LMW) forms. vWF is also a carrier for coagulation factor VIII (FVIII) and is essential for maintaining normal FVIII levels.

Slide 3:
von Willebrand disease is due to deficiency (quantitative) or dysfunction (qualitative) of vWF, or a combination of deficiency and dysfunction. The abnormalities result in defective platelet adhesion at sites of injury and a mucocutaneous bleeding pattern, which could include manifestations such as epistaxis, menorrhagia, or easy bruising. FVIII levels are also low in many cases. vWD is one of the most common inherited bleeding disorders and is usually transmitted in an autosomal dominant fashion, although there are also autosomal recessive forms. vWD can also be acquired with certain underlying medical conditions. The discussion today will focus on inherited forms of vWD.

Slide 4:

vWD is genetically and phenotypically heterogeneous. Type 1 vWD is characterized by deficiency of vWF, due to decreased vWF production, abnormal secretion, or increased degradation, and symptoms can vary from mild to severe, depending on the degree of deficiency. The protein that is present functions normally. Type 3 vWD is a severe bleeding disorder characterized by absence of vWF. The type 2 subtypes of vWD are caused by functional abnormalities of vWF due to an abnormal multimeric pattern (which can be due to problems with multimerization at the time of synthesis or increased clearance of larger multimers), abnormal platelet binding, abnormal collagen binding, or abnormal factor VIII binding. In type 2 subtypes, the dysfunctional vWF is also often present in reduced amounts. The mutations that cause type 2 forms of vWD are typically restricted to specific regions of the gene. For instance, forms with abnormal platelet binding have mutations in gene regions that code for platelet binding, such as exon 28.

Slide 5:
An initial hemostasis evaluation in a patient with a suspected bleeding disorder typically includes platelet count, prothrombin time (PT), activated partial thromboplastin time (aPTT), and often fibrinogen. The platelet count is normal in most forms of vWD, with the exception of type 2B and platelet-type (pseudo-vWD), which are characterized by gain-of-function mutations that cause increased vWF-platelet interaction and clearance of HMW multimers and platelets. The aPTT is often normal in vWD but is prolonged in more severe forms where FVIII activity is significantly decreased. Thus, vWD is in the differential for someone with a prolonged aPTT but this is not a sensitive way to identify all vWD cases. Type 2N vWD is characterized by decreased FVIII binding and may have normal vWF antigen levels, but with low FVIII, and a prolonged aPTT. This subtype often mimics hemophilia A (inherited FVIII deficiency). Point-of-care platelet function tests that may be performed as part of an initial work-up (such as the PFA-100 device) are usually normal in mild or moderate vWD and abnormal in more severe forms.

Slide 6:
If there is a strong suspicion of vWD based on the clinical history or initial laboratory tests (which may be normal), then a vWD testing panel should be performed. This should include measuring vWF antigen (performed by immunoassay), vWF activity, and FVIII activity. Because vWF has several functions, there are several different types of activity (functional) tests. One of the more common methods is known as ristocetin cofactor activity and will be described in more detail on the next slide. vWF multimeric analysis is an electrophoresis test that allows visualization of the size distribution of vWF multimers in patient plasma. It is used for subtyping of vWD since certain type 2 subtypes are associated with multimeric abnormalities due to missing high- or high- and intermediate- molecular weight multimers. The initial vWD testing panel is often sufficient for diagnosis and subtyping, but additional testing may be needed in some cases, and there are a number of specialized follow-up tests that can be pursued when indicated. These can include tests such as low-dose ristocetin-induced platelet aggregation (LD-RIPA) to detect gain-of-function vWF abnormalities, collagen binding activity, FVIII binding activity, or genetic testing. Genetic testing can be helpful for diagnosis of difficult type 2 vWD cases since type 2 mutations are located in specific regions of the gene corresponding to the affected vWF function. Sequencing of the entire vWF gene is not widely available and is done only rarely in current practice.

Slide 7:

This slide depicts the methodology for von Willebrand factor ristocetin cofactor activity (vWF:RCo), which is one of the most commonly used methods for assessing vWF activity. In this test, ristocetin is used to convert patient plasma vWF to its active platelet binding conformation. Agglutination of formalin-fixed reagent platelets is dependent on vWF amount, ability to interact with platelets through the platelet-binding domain, and presence of the larger, more functional multimers. The reagent is a turbid platelet suspension and patient vWF activity results in decreased turbidity as it causes platelet agglutination and settling of platelet agglutinates out of the reaction. There are also tests that measure vWF platelet binding activity without the use of ristocetin.

Slide 8:
Slide 8 contains an image of the vWF multimers test by gel electrophoresis. After the electrophoresis step, migrated vWF is visualized using an anti-vWF antibody and a second peroxidase-labeled antibody and specific substrate. Lane 1 is highlighted and demonstrates a normal multimeric distribution with the HMW multimers migrating near the point of application at the bottom of the gel, while the smaller multimers migrate further. Lane 2 shows an abnormal multimeric distribution with missing HMW multimers, which can be seen in certain type 2 subtypes (2A, 2B, platelet-type). Lane 10 shows a specimen with low vWF but the full spectrum of vWF multimeric sizes, typical of type 1 vWD.

Slide 9:
Type 1 vWD is the most common subtype, accounting for approximately 80% of the cases, and is characterized by a partial quantitative deficiency of vWF. The bleeding symptoms are often mild, but can also be moderate or severe, due to the genetic and phenotypic heterogeneity of the disease. The table and images on this slide show that in type 1 there is decreased vWF protein (as measured by vWF:Ag) and decreased activity (as measured by vWF:RCo in this example) due to the deficiency. Thus, in this subtype, the antigen amount and function are decreased proportionately and FVIII activity may also be low (which could result in a prolonged aPTT). The multimeric test shows decreased intensity of staining, correlating with the deficiency, but all multimer sizes are present. One of the challenges of diagnosing milder type 1 cases is the acute phase properties of vWF, which can raise vWF levels above the patient's normal baseline values and confound the diagnosis. In addition, because mildly decreased vWF values of approximately 30-50% of normal may be asymptomatic or associated with only minimal symptoms, these cases are often classified as "low vWF", but can be diagnosed as vWD if there is a strong clinical history. vWF values less than 20-30% of normal are generally associated with bleeding symptoms and diagnosed as vWD. Laboratories that perform vWD testing must handle specimens with care, especially when thawing frozen plasma for testing, as vWF can precipitate during the thawing process, inducing abnormalities that could lead to misdiagnosis.

Slide 10:
vWF is absent in type 3 vWD, which accounts for less than 5% of cases, and this results in a severe bleeding disorder. FVIII is also markedly decreased due to lack of vWF as a carrier protein, resulting in a prolonged aPTT in type 3 patients.

Slide 11:

The type 2 subtypes account for 10-15% of vWD cases and are defined by qualitative abnormalities (dysfunctional vWF) which can be due to multimeric defects where the larger, more functional multimers are absent, or loss-of-function mutations causing decreased platelet, collagen, or FVIII binding. These defects, along with associated subtypes, are listed on the slide. In type 2B and platelet-type, large multimers are absent due to increased interaction with platelets, resulting in clearance of large vWF and platelets. The bleeding symptoms are generally mild to moderate.

Slide 12:
As discussed on slide 9, type 1 vWD results from a vWF deficiency and results in concordant decreases in vWF antigen and activity. Because most type 2 subtypes demonstrate decreased platelet binding activity that is out of proportion to the vWF antigen level, due to missing large multimers or mutations specifically affecting the platelet binding domains, a decreased activity:antigen ratio raises suspicion of a type 2 subtype. As an example, a vWF:RCo to vWF:Ag ratio of less than 0.5 – 0.7 suggests dysfunctional vWF and prompts additional testing, such as multimeric analysis.

Slide 13:
Because a comprehensive review of each vWD subtype is outside of the scope of this presentation, type 2A will be presented in more detail as a type 2 example. This subtype is caused by a variety of mutations that result in decreased assembly/secretion or increased degradation of larger multimers. The multimeric defect, classically absence of both high- and intermediate- molecular weight multimers, can be seen by electrophoresis. Although the vWF antigen is decreased, smaller multimers are present and are measured antigenically. The missing large multimers, which are more functional than the smaller multimers, results in a disproportionate decrease in platelet binding activity as compared to the antigen.

Slide 14:
This slide depicts the laboratory data from a patient with type 2A vWD including the multimeric defect highlighted in position 5 on the gel and the discordant vWF activity and antigen resulting in a decreased activity:antigen ratio. With normal vWF function, the protein amount and activity would be similar. It should be noted that accurate subtyping of vWD is important since the subtype directs treatment choice. Treatment can include desmopressin (DDAVP) which releases endothelial vWF stores, vWF replacement with plasma derived or recombinant concentrates, vWF-containing blood products, and hormonal therapies to increase vWF levels. DDAVP is generally not used to treat type 2 (qualitative) subtypes of vWD, since releasing stores of abnormal vWF is unlikely to be helpful. DDAVP treatment can also worsen thrombocytopenia in type 2B due to acute release of vWF with an abnormally high affinity for platelets. When vWF replacement is required, specific vWF concentrates are preferred over blood products, which are only used when other treatment options are unavailable

Slide 15: References

Slide 16: Disclosures

Slide 17: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on "**von Willebrand Disease**."

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PEARLS OF LABORATORY MEDICINE

von Willebrand Disease

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DOI:

CCTC Clinical Chemistry Trainee Council

von Willebrand factor (vWF)

- Multimeric protein that mediates adhesion of platelets at sites of vascular injury
  - Collagen
  - Platelet glycoprotein Ib (GP1b) receptor
  - High-molecular-weight (HMW) multimers are more effective at binding platelets
- Carrier for coagulation factor VIII (FVIII)

von Willebrand disease (vWD)

- Deficiency (quantitative) and/or dysfunction (qualitative) of vWF
- Results in defective platelet adhesion and mucocutaneous bleeding pattern
- One of the most common inherited bleeding disorders
  - Usually autosomal dominant
- Rare acquired cases

vWD etiology

- Decreased production
- Abnormal secretion
- Increased degradation
- Abnormal multimeric pattern
- Abnormal platelet binding
- Abnormal collagen binding
- Abnormal FVIII binding

Quantitative: types 1and 3

Qualitative: type 2 subtypes

Initial hemostasis evaluation

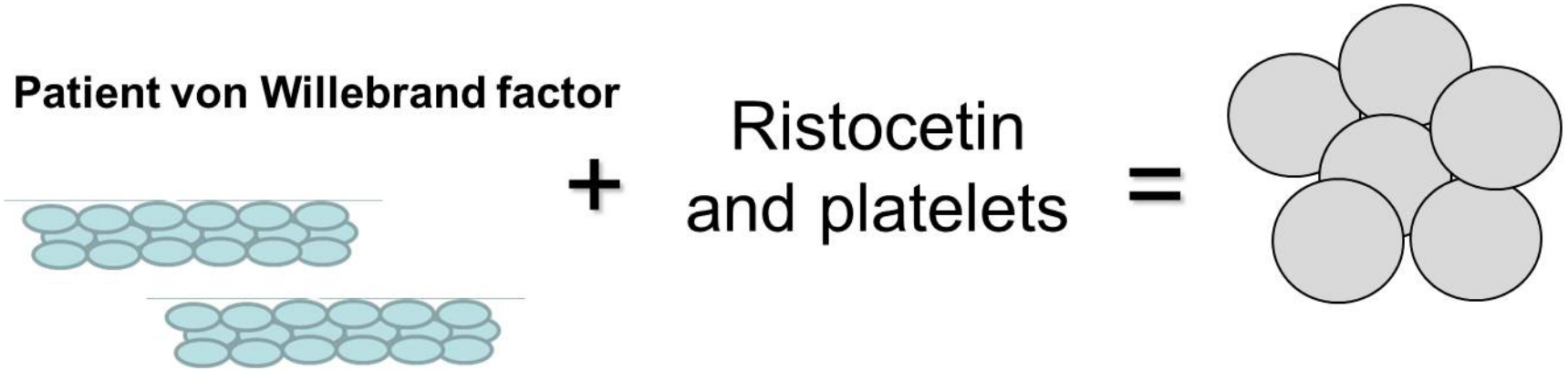
Test	Result in vWD
Platelet count	Usually normal
Prothrombin time (PT)	Normal
Activated partial thromboplastin time (aPTT)	Abnormal in severe vWD, often normal in mild/moderate vWD
Platelet function tests	Abnormal in severe vWD, often normal in mild/moderate vWD

Initial vWD evaluation

Test	Methodology
von Willebrand factor antigen (vWF:Ag)	Immunoassay
von Willebrand factor activity (Ristocetin cofactor activity, vWF:RCo)	Platelet agglutination
Factor VIII activity	Clot-based (aPTT)
Multimeric analysis	Gel electrophoresis; used for vWD subtyping; shows presence and relative concentration of various sizes of multimers

vWF:RCo

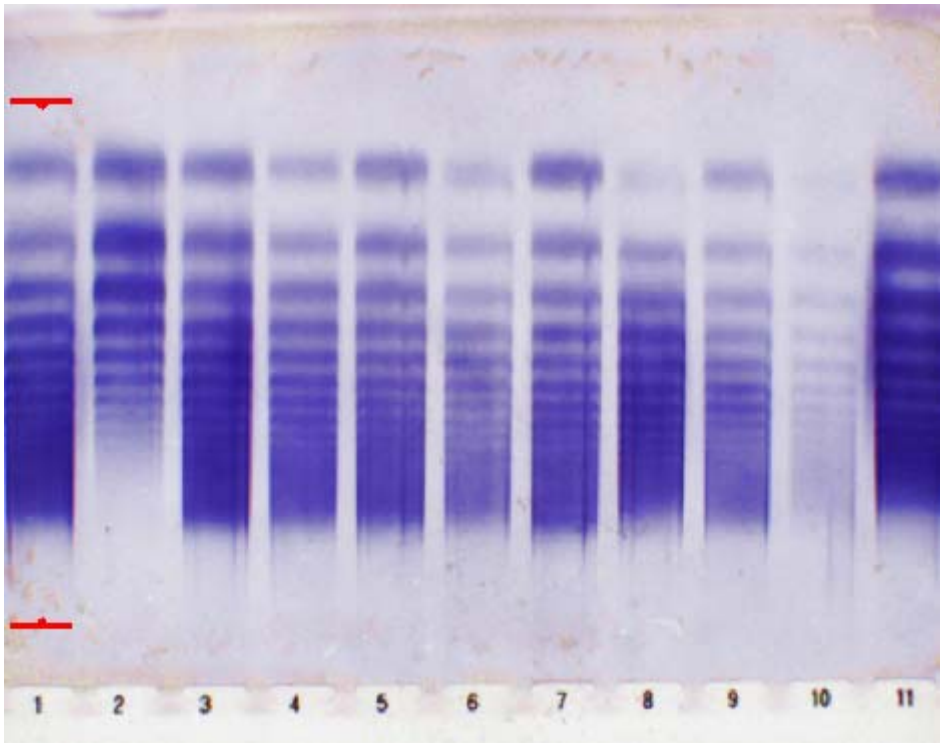
- Platelet agglutination method
  - Ristocetin causes patient HMW vWF to bind and agglutinate reagent platelets, decreasing turbidity



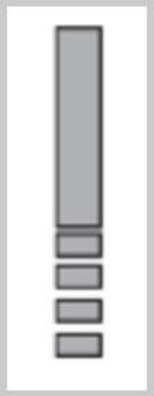
Multimeric analysis

LMW multimers

HMW multimers



Type 1

Test	Result
vWF:Ag	Decreased (variable severity)
vWF:RCo	Decrease proportionate to vWF:Ag
RCo:Ag Ratio	Normal (close to 1)
FVIII	Normal or decreased
Multimer Example: Normal multimer 	Normal 

Type 3

Test	Result
vWF:Ag	Absent
vWF:RCo	Absent
FVIII	<10% of normal
Multimer	Absent

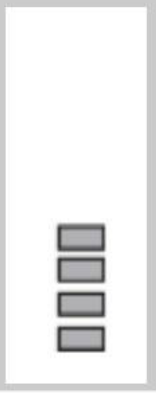
Type 2 subtypes

- Qualitative (protein functions abnormally)
- Mutations affect interaction with ligands
  - Missing large multimers (HMW and/or IMW)
    - 2A, 2B, platelet-type
  - Decreased platelet or collagen binding
    - 2M
  - Decreased FVIII binding
    - 2N

Type 2 – use of activity to antigen ratio

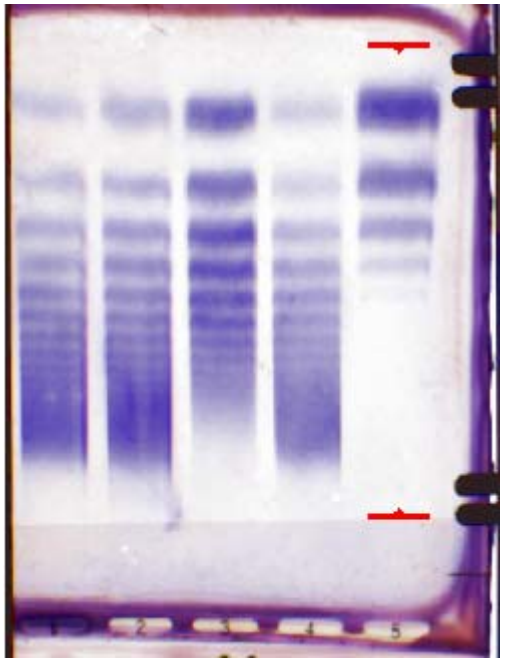
- Majority of type 2 cases (except 2N, some cases of 2M) demonstrate decreased platelet binding activity
  - Missing large multimers
  - Loss of function mutation affecting platelet binding domain
- Results in decreased activity:antigen ratio (such as RCo:Ag ratio) (< 0.5 - 0.7)

Example: Type 2A

Test	Result
vWF:Ag	Mild decrease
vWF:RCo	Moderate to severe decrease
RCo:Ag Ratio	Decreased
FVIII	Normal or decreased
Multimer Example: Normal multimer 	Missing HMW and IMW multimers 

Example: Type 2A

Test	Result	Reference Interval
vWF:Ag	46%	52-214%
vWF:Rco	<10%	51-215%
Rco:Ag Ratio	<0.2	>0.5
FVIII	60%	56-191%
Multimer	HMW/IMW multimers absent	Normal



References

1. Nichols WL, Hultin MB, James AH, et al. Von Willebrand disease (vWD): evidence-based diagnosis and management guidelines, the National Heart, Lung, and Blood Institute (NHLBI) Expert Panel report (USA). Haemophilia 2008;14:171-232
2. Sharma R and Flood VH. Advances in the diagnosis and treatment of Von Willebrand disease. Blood 2017;130(22):2386-91.
3. Roberts JC and Flood VH. Laboratory diagnosis of von Willebrand disease. Int Jnl Lab Hem 2015;37 (Suppl. 1): 11-17.

Disclosures/Potential Conflicts of Interest

Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

- **Employment or Leadership**:
- **Consultant or Advisory Role:**
- **Stock Ownership:**
- **Honoraria:**
- **Research Funding:**
- **Expert Testimony:**
- **Patents:**

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Question Bank
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Field	Instructions	
Stem	Write one question *Refer to Guide for Presenters for guidance (Page 5)*	Which of the following is true regarding laboratory testing for von Willebrand disease (vWD)?
Responses	Provide 5 responses *Refer to Guide for Presenters for guidance (Page 5)*	A- Activity and antigen measurements are always similar B- Genetic testing is required for definitive diagnosis C- Multimeric analysis is used for subtyping D- The activated partial thromboplastin time is always prolonged E- vWF values slightly below the reference interval are diagnostic of vWD
Answer	Indicate one correct response	C
Discussion	Provide a discussion of the correct response with main points explaining why it is the best choice	von Willebrand factor multimeric analysis shows the presence and relative concentration of various sizes of multimers and is used to assist with vWD subtyping since certain subtypes demonstrate multimeric abnormalities
Source(s)	Provide the source(s) of information for further study *Refer to Guide for Presenters for full citation formatting (Page 3)*	Roberts JC and Flood VH. Laboratory diagnosis of von Willebrand disease. Int Jnl Lab Hem 2015;37 (Suppl. 1): 11-17.
Difficulty	Select one level of difficulty: *Easy, intermediate, advanced*	Intermediate
Category	Select one category (*Refer to list in Guide for Presenters - Page 6)*	Coagulation
Sub-category	Select one sub-category (*Refer to list in Guide for Presenters - Page 6)*	Coagulation

Keywords	Include at least 1-2 keywords *Keywords should describe a subtopic to the sub-category selected. Examples include, thyroid, electrolytes, diabetes, pregnancy, etc.*	Coagulation, von Willebrand disease
Field	**Instructions**	
Stem	Write one question *Refer to Guide for Presenters for guidance (Page 5)*	Which of the following is true regarding type 1 von Willebrand disease (vWD)?
Responses	Provide 5 responses *Refer to Guide for Presenters for guidance (Page 5)*	A- It is characterized by dysfunctional von Willebrand factor B- Most patients have a severe bleeding phenotype C- Test results show concordant decreases in vWF antigen and activity D- Type 1 is a rare subtype E- vWF multimers are abnormal
Answer	Indicate one correct response	C
Discussion	Provide a discussion of the correct response with main points explaining why it is the best choice	Type 1 vWD is the most common subtype and is due to a vWF deficiency which is seen in lab tests as concordant decreases in vWF antigen and activity with a normal multimeric distribution. The bleeding phenotype is variable but most type 1 patients have a mild bleeding disorder.
Source(s)	Provide the source(s) of information for further study *Refer to Guide for Presenters for full citation formatting (Page 3)*	Nichols WL, Hultin MB, James AH, et al. Von Willebrand disease (vWD): evidence-based diagnosis and management guidelines, the National Heart, Lung, and Blood Institute (NHLBI) Expert Panel report (USA). Haemophilia 2008;14:171-232
Difficulty	Select one level of difficulty: *Easy, intermediate, advanced*	Intermediate
Category	Select one category (*Refer to list in Guide for Presenters - Page 6)*	Coagulation
Sub-category	Select one sub-category (*Refer to list in Guide for Presenters - Page 6)*	Coagulation

Keywords	Include at least 1-2 keywords *Keywords should describe a subtopic to the sub-category selected. Examples include, thyroid, electrolytes, diabetes, pregnancy, etc.*	Coagulation, von Willebrand disease