Laboratory Diagnosis of von Willebrand Disease

Larry Smith, PhD, SH(ASCP), HCLD/CC(ABB)

Medical and Scientific Affairs, Liaison Manager
ABBOTT Diagnostics – Hematology
Santa Clara, CA  95054
Larry.Smith@abbott.com
DISCLOSURE STATEMENT

Nothing to disclose

1. Medical and Scientific Affairs, Abbott Diagnostics –Hematology
2. Lecture for ASCP
3. Adjunct Professor, Rutgers University, NJ
4. Prior to joining Abbott: Director Coagulation Laboratory, Assistant Director Hematology and Flow Cytometry, Memorial Sloan Kettering Cancer Center, New York

CONTENT OF PRESENTATION IS CONSISTENT WITH ALL APPLICABLE FDA REQUIREMENTS.
Objectives

- Define von Willebrand Disease
- Describe current classification of VWD
- Review structure and function of VWF
- Discuss methods for diagnosing VWD and assay limitations and preanalytical variables influencing VWD testing and interpretation
VWD—Disorder of Primary Hemostasis

• Most common of the \textit{congenital} bleeding disorders
  - 1-2\% of the general population
• Symptomatic in only about 1/10,000
- 1926 – Erik von Willebrand $\rightarrow$ 5 y-o-f and her family who lived on the Åland Islands – \textit{Hereditär pseudohemofili, 1926}
- Initially described as “pseudohemophilia”
The Coagulation Cascade

Surface, XII
Prekallikrein
HMWK

Central Player
- Converts Fibrinogen to Fibrin
- Platelet activation
- FV, VIII, XI
- FXIII

Tissue Factor
VII(VIIa)

Thrombin

- Converts Fibrinogen to Fibrin
- Platelet activation
- FV, VIII, XI
- FXIII

Fibrinogen

Fibrin

Stable Clot

Monomers
Primary Hemostasis

• Involves the interaction of
  - Platelets
  - Blood vessels

• Leads to initial clot formation → primary hemostatic plug
Secondary Hemostasis

Reinforcement of the platelet plug by fibrin formation via activation of the coagulation factors
Naturally Occurring Inhibitors of the Coagulation Cascade

1. TFPI
2. AT
3. PC/PS → aPC
4. TM

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VWD—Disorder of Primary Hemostasis

• Clinical manifestations
  - Mucocutaneous bleeding of varying severity in males and females
    • Ecchymoses
    • Epistaxis
    • Gastrointestinal bleeding
    • Menorrhagia

• Bleeding in VWD is due to
  • Defective platelet adhesion
  • Reduced FVIII levels
VWF

- Large multimeric protein – ranges from 600 to >20 million Daltons
  - Gene for VWF is located on chromosome 12p
    - 178 kB, 52 exons
    - Pseudogene
    - cDNA

Synthesis of vWF

- VWF synthesized in endothelial cells and megakaryocytes
  - Stored in Weibel-Palade bodies of endothelial cells
  - Stored in α-granules of platelets

- Steps in synthesis of VWF
  - *Pre-pro-VWF monomer* in nucleus
  - *Signal peptide is cleaved*
  - *Dimerization* occurs in ER
    - *Linked together* at the carboxyl terminal end
  - Dimers *multimerize* in the Golgi
  - Propeptide is *cleaved* off → *mature* subunit

*Image of VWF synthesis*

*Blood. 2015;126(15):1753-61*
VWF Release


*Image of VWF coiling and release from Weibel-Palade bodies*

• *Image of VWF cleaving protease (ADAMTS13)*

Function of vWF

VWF serves **two** important biologic functions

1. Serves as a ligand that binds to the **gpIb** receptor on platelets to initiate platelet **adhesion** to the damaged endothelium
   - VWF binds to **extravascular collagen**
   - Platelets adhere to the bound vWF
   - Adherent platelets become activated
Function of vWF

VWF serves **two** important biologic functions

2. Serves as a carrier protein for plasma FVIII
   - VWF protects Factor VIII in circulation
   - VWF co-localizes FVIII at sites of vascular injury

![Diagram of vWF function](image-url)

- **Endothelium**
- **Subendothelial collagen**
- **FVIII**
- **PLT**
- **VWF**
Function of vWF

VWF interacts with clotting factors, platelets, and the vessel wall

Diagram:
- Platelets
- Clotting factors
- Vessel wall

vWF interacts with Janus-faced protein
How do we classify vWD?

Essential assays to classify vWD?
Essential Assays for VWD

• Screening assays
  • FVIII:C
  • VWF antigen assay
  • VWF activity assay

• Platelet Function Screen (BT or PFA)

• Multimer analysis
• Platelet aggregation
Classification of vWD

• VWD – extremely heterogeneous, complex disorder with > 20 distinct subtypes

• Two broad categories

1. **Quantitative** Defects
   • Type 1
     • Partial quantitative deficiency
   • Type 3
     • Complete absence/severely decreased

2. **Qualitative** Defects
   • Type 2
     • 2A
     • 2B
     • 2M
     • 2N

Subgroups
Quantitative Defects

Normal Protein

Decreased amount of protein
Type I vWD

- **Most common** type of VWD
  - 80% of patients with VWD fall into this category

- Caused by heterozygous mutation leading to a **partial quantitative deficiency** of vWF
  - Genetic abnormality in ONE of the VWF alleles
    - Accounts for a 50% reduction in VWF
    - Mild secondary deficiency in FVIII

- Bleeding symptoms range from **asymptomatic to mild**

- Endothelial cells and platelets contain **normal**, but **reduced** levels of VWF
  - DDAVP can induce the release the **stored** VWF
Type I vWD

- Laboratory findings
- Normal to decreased
  - FVIII (aPTT)
  - VWF:Activity (Ristocetin Cofactor)
  - VWF:Antigen

- Prolonged BT
  - (PFA-100/200—prolonged CT)
- Proportional decrease of ALL VWF multimers
Type 3 vWD

- **Most severe** form of the disease

- Results from a **homozygous** mutation leading to a deficiency of vWF with **absent or profound deficiency** in levels of plasma VWF

- Autosomal recessive

- VWF levels are <5%
  - FVIII is markedly cleared from the plasma with levels below 5-10%
  - FVIII is **not** as severely depressed as in severe Hemophilia A
  - Spontaneous bleeding
  - Severe mucocutaneous bleeding
  - Soft tissue/musculoskeletal bleeding

- 1-5% of case
  - Prevalence increases in regions of consanguineous marriages
Qualitative Defects

- **Abnormal** Protein
- Normal/decreased amount of protein
Type 2A vWD

- Mutations commonly occur in the A2 domain
- **LOSS** platelet-dependent function

- Only the smaller VWF multimers in plasma are present
- **Two proposed mechanisms:**
  - Abnormal assembly and secretion of large VWF multimers
  - Increased susceptibility of VWF to proteolysis in circulation

- Patients exhibit moderate to severe mucocutaneous bleeding
Type 2B

- Mutation in the **A1 domain of the VWF gene**

  - "**Gain-of-function**" mutation in VWF → increased affinity to bind to the gpIb platelet receptor
    - Spontaneous binding to platelets
    - Rapid clearance of the large multimers
    - Thrombocytopenia

- **DDAVP contraindicated** → would cause increased thrombocytopenia as platelets would be hyper-reactive to the released VWF
Type 2M vWD

- Mutations in exon 28 in A1 domain

- Defect leads to decreased or absent binding of VWF to platelet GPIb receptor
  - Decreased platelet dependent function
  - Plasma binding to FVIII is normal
  - Normal multimer profile
Type 2N vWD

- Also referred to as “autosomal hemophilia” or the Normandy variant
- Caused by mutations in the FVIII binding domain of VWF
- Markedly decreased affinity for binding to FVIII → rapid turnover of the unbound FVIII

Lab findings
1. Decreased FVIII
   - Similar to “mild” hemophilia
2. Normal VWF antigen and activity
3. Platelet binding to vWF is normal
   - Normal bleeding time (PFA-100/200)

Genetic counseling and treatment is different from hemophilia
Pseudo-von Willebrand Disease – (Platelet type vWD)

• VWF molecule is NORMAL

• “Gain-of-function” mutation in the platelet gpIb receptor
  • Increased affinity of platelets for VWF
  • Enhanced clearance of VWF and platelets from circulation

• Lab findings
  - Loss of high molecular weight multimers
  - Platelet count is low**
  - Platelet aggregation with low dose ristocetin (RIPA)

• Defect is in the platelet → standard approaches to treating VWD are not helpful
Vicenza

- AKA type 1C VWD
- Characterized by *low plasma levels of VWF* due to increased clearance of VWF from the plasma
  - VWF levels ~6-10 IU/dL and ultra-large multimers are present

- Lab findings
  1. Low VWF activity and antigen
  2. FVIII may be low
  3. *Elevated VWF propeptide* to VWF antigen ratio
  4. Persistence of larger than normal size multimers
  5. Increased rate of VWF clearance in DDAVP trials

- Unclear if the decreased function is a *quantitative* problem or a “true” functional abnormality

- Assay principle
  - PCR amplification and bi-directional DNA sequence analysis
Vicenza

Image of propeptide, endothelial cells and mature VWF protein in VICENZA

From: ASH Education book 2012, Branchford, B, Di Paolo, J
Acquired vWD

• Acquired qualitative, structural, or functional disorder
  • Increased risk of bleeding

• Mechanisms
  1. Autoimmune clearance/inhibition of VWF
     • Lymphoproliferative, MGUS, SLE
     • Autoantibodies → increased clearance of VWF from plasma
  2. Hypothyroidism – nonimmune mechanism
  3. Fluid shear stress-induced proteolysis – AVS, LVAD
     • Increases proteolysis by ADAMTS13 → depletes large VWF multimers
  4. Increased binding to cell surfaces – ET
  5. Drugs – Valproic acid, ciprofloxacin, griseofulvin

• Laboratory
  - Activity and antigen often do not match
Assays for vWD
Assays for VWD

• Screening assays
  • Platelet Function Screen (BT or PFA)
    • FVIII:C
    • VWF antigen assay
    • VWF activity assay
      • Ristocetin cofactor
      • LIA-assays
      • Collagen-binding assay

• Confirmatory Assays
  • Multimer Analysis
  • Ristocetin-induced platelet aggregation
Assays for VWD

PFA-100 (Bleeding time)

- Combined sensitivity to Types 1, 2A, 2B, 2M, 3 ~ 85-90%
- Sensitivity to Type 2A, 2B, 2M, 3 >98%
Assays for vWD

- **VWF:Antigen**
  - Immunoassay that measures the *concentration of VWF protein* in plasma
  - Detects all forms of VWF (*functional and nonfunctional forms*)
  - Cannot discriminate between multimer size
VWF Activity Assay Principle

Normal platelets → VWF (Patient) → Platelet agglutination

Change in OD
Assays for vWD

• vWF:Activity
  - **Ristocetin cofactor assay** *(gold standard)*
    • Measures the ability of VWF (patient) to induce agglutination of normal fixed platelets in the presence of **Ristocetin**
    • *Mix patient’s plasma + normal donor platelets + ristocetin → platelet agglutination reaction occurs on platelet aggregometer*

CV’s – 10-30%
Assays for vWD

• vWF:Activity

  • Latex particle enhanced immunoturbidimetric assay
    - *Specific anti-VWF monoclonal antibody*
      • Directed against the platelet binding site on VWF (gp Ib receptor)
      • Degree of agglutination directly proportional VWF activity

    • Mix patient’s plasma + latex beads coated with an anti-VWF monoclonal antibody ➔ agglutination of beads

Ab-coated beads

Add patient plasma (VWF)

Ag:Ab binding
Assays for vWD

- vWF:Activity (1) — NO ristocetin added
  - Immunoturbidimetric assay that uses a recombinant form of the GPIIbα receptor containing two gain-of-function mutations
  - An antibody against the GPIb-α captured onto polystyrene particles
  - Gain-of-function mutation induces binding in the absence of ristocetin
  - Binding occurs → change in turbidity

- vWF:Activity (2) — Ristocetin added
  - Immunoturbidimetric assay that uses a recombinant fragment of the GPIIbα receptor
  - Ristocetin induces binding of the monoclonal antibody to VWF which leads to agglutination
    - \([\text{Latex particle} + mAB + rGPIb\alpha + \text{ristocetin}] \rightarrow \text{change in OD}\)
**Assays for vWD**

- **FVIII**
  - Clot-based assay that measures level of circulating of FVIII
  - Patient plasma (unknown level FVIII *fairly prolonged clotting time*) + Normal Plasma (deficient in FVIII *extremely prolonged clotting time*)
  - Correction of the clotting time of the deficient plasma using *patient plasma*
  - Compare CT to calibration curve
    - CT’s established using known conctrs of factor
  - Report in IU/mL or % of normal
Assays for vWD

- **Multimer Analysis**
  - *Qualitative assay* (electrophoresis) to depict the *variable concentrations* of different-sized VWF multimer
    - **Separation** of band on agarose gel
      - High, medium, or low resolution
      - Low resolution (<1.0% agarose) superior for separating the *largest* VWF multimers
      - Higher resolution (>1.0% agarose) superior for separating *triplet structures*
    - **Fixation** with antibody specific for VWF
    - **Transfer** to nitrocellulose, nylon or polyvinylidene difluoride
    - **Visualization** → immunologically by colorimetry, chemiluminescence or fluorescence
    - **Quantification** → densitometer

Assays for VWF

- Collagen Binding Assay
  • Measures the ability of large VWF multimers to bind collagen
  • Can be used to differentiate between Type 1, Type 2A, Type 2B and Type 3 VWD in which there is a loss of high molecular weight multimers
  • More sensitive than VWF-RCO to the loss of the highest multimers

1. Standardization?
2. Different sources of collagen
3. FDA approval?
Additional Assays

- Ristocetin Induced Platelet Aggregation (*RIPA*)
  • Measures the ability of patient’s VWF and patient’s platelet gpIb receptor to aggregate in the presence of ristocetin
  • Increased in Type 2B VWD and platelet-type VWD

Ratios in VWD Testing

• To differentiate between VWD type 1 and 2

- **VWF:ACT/VWF:AG**
  • >0.7  \(\rightarrow\) Type 1
  • <0.7  \(\rightarrow\) Type 2

- **VWF:CB/VWF:AG**
  • <0.7  \(\rightarrow\) Type 2

- **FVIII/VWF:AG**
  • Decreased  \(\rightarrow\) 2N (or hemophilia A)
**Additional Assays**

- **VWF Platelet Binding Assay**
  - Measures the *ability of VWF to bind to normal formalin-fixed platelets* in the presence of a *low dose* of ristocetin
  - Differentiate *Platelet-type vWD from Type 2B vWD*

*Increased binding = consistent with type 2B VWD*

(defect in the patient’s plasma since we are using normal donor platelets)

*NO binding = consistent with platelet-type vWD*

Normal donor Platelets
Additional Assays

• VWF FVIII-Binding Assay
  - Measures the ability of vWF to bind to recombinant FVIII
  • Type 2N has decreased binding to FVIII
VWF Propeptide assay

• VWF propeptide is synthesized as part of the VWF molecule

![Diagram of VWF molecule showing propeptide and its components](image)

• Propeptide is stored along with mature VWF
  1. If VWF synthesis is low, then the propeptide level is low
  2. If VWF antigen is decreased but the propeptide levels are normal, suggest increased clearance
    • 1C, 2A, 2B, acquired

• Often used in response to Desmopressin to identify increased clearance
  - Antigen is increased after 1 hour but falls back to baseline in 4 hours
  - Propeptide remains normal

• Published range = 55-291 U/dL -- Report as a ratio of VWF:PP/VWF:AG

**DDAVP Challenge**
Genetic Testing in VWD

• Genetic testing for VWD is not indicated except for specific cases in which the results of the test would make a difference in the patient's therapeutic management or counseling.

• There are several complicating factors that make genetic testing difficult for VWD:
  - *VWF* is a very large gene that spans 178 kb and contains 52 exons.
  - The presence of the highly homologous partial pseudogene on chromosome 22 makes sequencing and interpretation particularly difficult.
  - The gene is also highly polymorphic with >300 single nucleotide polymorphisms reported.
Preanalytical Variables
Collection and Storage

• Sample collection and handling
  - Phlebotomy conditions
  - Patient’s stress level
  - Sample processing
  - Sample storage
    • Refrigeration/storage of citrated blood > 4 hours → artifactually low VWF levels and loss of HMWM’s
    • Bohm, et al, Blood Coagulation Fibrinolysis 2006:17, No.1

Image showing VWF protein deterioration and VWF multimer degradation as a result of being placed on ice

Bohm, et al, Blood Coagulation Fibrinolysis 2006:17, No.1
Race and Blood Groups

- Race
  • Higher levels seen in African/African-Americans

- Age
  • Levels increase with age

- ABO Blood Groups

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<th>Mean</th>
<th>Mean +/- 2SD</th>
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<td>74.8</td>
<td>35.6 – 157.0</td>
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<tr>
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<td>240</td>
<td>105.9</td>
<td>48.0 – 233.9</td>
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<tr>
<td>B</td>
<td>196</td>
<td>116.9</td>
<td>56.8 – 241.0</td>
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<tr>
<td>AB</td>
<td>109</td>
<td>123.3</td>
<td>63.8 – 238.2</td>
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Adapted from Gill et al in Blood 987;69:1691
Miscellaneous Variables

- Conditions with **elevated** VWF levels
  - Age
  - Acute and chronic inflammation
  - Diabetes
  - Malignancy
  - Pregnancy or OCT
  - Stress, exercise
  - Hyperthyroidism

- Condition with **reduced** VWF levels
  - Hypothyroidism
  - Type O blood
“Low” VWF versus VWD

• Low VWF levels is diagnostic of type 1 VWD
  
  - However some patients with VWF levels between 30-50 may have “Low” VWF rather than VWD
  
  • Many diagnoses of VWD type 1 are actually FALSE positives due to low VWF levels
  
  • True VWD should probably have levels < 30%

  • Levels are between 30-50% and present with mild mucocutaneous bleeding – might suggest VWD rather than low VWF

  • Risk of bleeding increases as the VWF levels decreases → relationship is not strong until VWF levels are very low
Treatment of von Willebrand Disease

1. DDAVP (*deamino-8-arginine vasopressin*)
   - Synthetic analogue of the natural pituitary hormone
   - Releases *intracellular VWF* from endothelial cells → raises plasma levels of VWF
   - Treatment of choice in Type I
     - Variable response in 2A and 2M
     - Ineffective in Type 3
     - Contraindicated in 2B
   - Can also be given as an inhaler (nasal spray)
Treatment of von Willebrand Disease

2. Humate-P – contains FVIII and VWF (large multimers) – FDA-approved
   - Alphanate, Koate

3. ε-Aminocaproic Acid (EACA) and Tranexamic Acid
   - Fibrinolysis inhibitors

4. Cryoprecipitate
   - Source of fibrinogen, factor VIII, VWF and FXIII
   - Only plasma fraction that consistently contains VWF multimers
   - *USE WITH CAUTION!!!*
Case Studies
Case 1

• 18 y-o-f presents with easy bruising, on all 4 extremities, heavy menses, prolonged bleeding after wisdom tooth extraction

• Lab values
  - PT = 12.0 sec (9.5 – 12.8 sec)
  - aPTT = 38.0 sec (24.0 – 36.3 sec)

  - FVIII = 40% (50 – 150%)
  - FIX = 105 % (50 – 150%)
  - FXI = 113% (50 – 150%)

  - VWF:Act = 35% (50 – 150%)
  - VWF:Ag = 37 % (50 – 150%)
  - ACT/AG = 0.9

Type 1
Case 2

- 32 y-o-f presents in 2\textsuperscript{nd} trimester pregnancy. She had excessive bleeding after arthroscopy. She has no reported history of excessive bruising in the past. She has a brother who died from bleeding at 12 years of age after an appendectomy.

- Lab values
  - PT = 11.5 sec (9.5 – 12.8 sec)
  - aPTT = 59.0 sec (24.0 – 36.3 sec)
  - FVIII = 11\% (50 – 150\%)
  - FIX = 115 \% (50 – 150\%)
  - FXI = 78\% (50 – 150\%)
  - VWF:Act = 69\% (50 – 150\%)
  - VWF:Ag = 73 \% (50 – 150\%)

Type 2N

Hemophilia?
Why no bruising?
Why are the activity and antigen normal?
Could pregnancy be a factor?
Case 3

• 51 y-o-m with HCV, extensive cardiac history and recent aortic stenosis presents with thrombocytopenia and a mild coagulopathy

• No reported history of excessive bleeding or bruising in the past

• Lab values
  - PT = 12.2 sec (9.5 – 12.8 sec)
  - aPTT = 46.0 sec (24.0 – 36.3 sec)
  - FVIII = 163% (50 – 150%)
  - FIX = 56% (50 – 150%)
  - FXI = 26% (50 – 150%)
  - VWF:Act= 72% (50 – 150%)
  - VWF:Ag = 162% (50 – 150%)
  - ACT/AG = 0.4

Acquired

How do you explain the bleeding?
Case 4

- 35 y-o-f presents with hyper-menorrhagia for 14 days and no previous bleeding history
- Epistaxis and ecchymosis noted
- Easy fatigability, decreased appetite
- Negative history of bleeding and no family history of bleeding

- Lab results:
  - PT = 12.6 sec (9.5-12.8)
  - APTT = 58.0 sec (24.0 – 36.3 sec)
  - VWF:AG = 18 (50-150)
  - VWF:ACT = 14 (50-150)
  - FVIII = 24% (50-150)

What is abnormal?

Type of bleeding? 1º or 2º

Acquired
Case 5

- 16-y-o-f is referred to the hematology department with a 3 year history of easy bruising and occasional episodes of epistaxis
- Her father had epistaxis as a child

- Lab values:
  - PT = 15.2 sec (9.5-12.8)
  - APTT = 36.0 sec (24.0-36.3)
  - VWF:AG = 38% (50-150)
  - VWF:ACT = 34% (50-150)
  - FVIII = 60% (50-150)
  - COL/EPI and COL/ADP = >240 sec
  - Multimer analysis = normal
  - Blood type: O+

Type 1
Case 6

• A 55-y-o-m comes to the ED with epistaxis. He reports that he has “bleeder’s disease” and has had multiple episodes of hemarthrosis (joint bleeding) and that his brother suffers from the same type of bleeding.

• Lab values:
  - PT = 11.1 sec (9.4-12.8)
  - APTT = 49.5 sec (24.0-36.3)
  - VWF:AG = 48% (50-150)
  - VWF:ACT = 45% (50-150)
  - FVIII = 18% (50-150)
  - Blood type = O+
Case 7

• 49-y-o-m presents to the clinic with lower back pain that has been present for the past 4 weeks with increasing intensity over the past 2 weeks.
• Current diagnosis of ET

Lab values:
• PT = 10.5 sec (9.5-12.8)
• aPTT = 48.0 sec (24.0-36.3)
• FVIII = 7% (50-150)
• FIX = 121% (50-150)
• vWF:ACT = 39% (50-150)
• vWF:AG = 51% (50-150)
• PLT CT = 1.250 x 10^9/L (180-410)
Case 8

• 3 y-o-m was referred to the pediatric hematology service for prolonged oral bleeding following an injury to his face. A number of bruises were noted during the time of examination.

• The family reported to prolonged bleeding in the past but no family history of bleeding.

• Laboratory values:
  - Normal platelet count
  - PT = 12.2 sec (9.5-12.8)
  - APTT = 69.0 sec (24.0-36.3)
  - vWF:ACT = 9% (50-150)
  - vWF:AG = 7% (50-150)

Type 3

Would DDAVP be appropriate?

What would his FVIII be?
Case 9

- 24 y-o-f with a family history of bleeding was referred by her OB/GYN to the hematology service for consultation and bleeding management post delivery
- She was reported to have prolonged PFA-100 results and a normal platelet count, normal PT and aPTT
- Further studies
  - Repeat PFA-100 = prolonged
  - Normal platelet count
  - PT and APTT WNL
  - VWF:ACT = 19% (50-150)
  - VWF:AG = 48% (50-150)
- Platelet aggregation
  - Col = normal
  - ADP = normal
  - AA = normal
  - Risto HD = normal
  - Risto LD = increased
Case 9

- VWD? Yes

Type of VWD? Type 2B vs Platelet-type VWD

- Multimer pattern? Loss of High in Intermediate multimers

- How to differentiate? Mixing study or genetic studies

DDAVP? No!!!
Case - 9

• How can you differentiate between 2B- and platelet-type VWD?

RIPA Mixing Study
Normal plasma + patient plasma

• Type 2B
  - Patient plasma with normal donor (control) platelets
    • NO correction in the aggregation pattern – defect in plasma

• Platelet-type
  - Patient plasma with normal donor (control) platelets
    • Correction in the aggregation pattern since the defect is in the platelet

DNA sequence analysis
  1. Exon 28 /VWF gene
  2. Platelet GP1BA gene
Case 10

- 7 y-o-m presents to the ED with uncontrolled epistaxis, multiple ecchymoses and gingival bleeding. He was admitted for observation to the pediatric ward.

- The following laboratory values were obtained:
  - WBC = 9.7 x 10⁹/L (4.2-10.0)
  - HGB = 8.3 g/dL (13.8-16.0)
  - PLTCT = 112 x 10⁹/L
  - MPV = 14.6 (7.0-12.0)
  - PT = 11.1 sec (9.5-12.8)
  - APTT = 35.0 sec (24.0-36.3)
  - vWF:ACT = 219% (50-150)
  - vWF:AG = 242% (50-150)

http://www.bloodmed.com/home/slide-popup.asp?id=560
Case 10

• Describe the PB film
  - Thrombocytopeia with large/giant pLTS

• What type of bleeding pattern do we see?
  - Primary hemostatic defect

• Could the bleeding be due to a disorder of secondary hemostasis?
  - Probably not

• Could the bleeding be due to VWD? What type?
  - Yes. 2B or pseudo VWD or something else!!!!

• How do you explain the elevated VWD? Are these values real?
  - Acute phase (child crying) Suggest repeat when the child is calm.

• What follow-up tests would you consider suggesting to the attending physician?
  - Platelet Aggregation and/or flow cytometry

http://www.bloodmed.com/home/slide-popup.asp?id=560
Case 10 – What if there were no response to Ristocetin? What would the differential diagnosis be?

Hi-dose Ristocetin

Differential Diagnosis if no response to Ristocetin?

http://www.anmjournal.com/article.asp?issn=0331-3131;year=2012;volume=6;issue=1;spage=35;epage=40;aulast=Belurkar

BS or vWD
Case 10

• Flow cytometry was performed

Is CD41 present?

Is CD61 present?

Diagnosis?

BS
Case 10

Platelet Aggregation in Normal Individuals and in Glycoprotein Disorders

http://www.mlo-online.com/clinical-perspectives-on-platelet-function-testing.php
## Interpreting Lab Data

<table>
<thead>
<tr>
<th>Lab Test</th>
<th>Type 1</th>
<th>Type 2A</th>
<th>Type 2B</th>
<th>Pseudo</th>
<th>Type 2M</th>
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<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>N</td>
<td>↑↑↑</td>
</tr>
<tr>
<td>FVIII</td>
<td>N to ↓</td>
<td>N to ↓</td>
<td>N to ↓</td>
<td>N to ↓</td>
<td>N</td>
<td>↓↓↓↓</td>
<td>↓↓↓↓</td>
</tr>
<tr>
<td>vWF:AG</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓ or (N)</td>
<td>↓ or (N)</td>
<td>↓ or (N)</td>
</tr>
<tr>
<td>RCOFTR</td>
<td>↓</td>
<td>↓↓↓↓</td>
<td>↓↓↓↓</td>
<td>↓↓↓↓</td>
<td>↓ or (N)</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>RIPPA</td>
<td>None</td>
<td>None</td>
<td>↑</td>
<td>↑</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Multimers</td>
<td>N but ↓</td>
<td>Abnormal</td>
<td>Abnormal</td>
<td>Abnormal</td>
<td>N but ↓</td>
<td>N but ↓</td>
<td>ABSENT</td>
</tr>
</tbody>
</table>

### Levels:
- **High**
- **Intermed**
- **Low**
Diagnostic Algorithm

Disorders of VWF Production/Clearance

- If severely low levels of VWF:Ag
  - VWD Type 3

- VWF:RCo/VWF:Ag > 0.6
  - VWD Type 1

- If elevated VWF:PP/VWF:Ag
  - VWD Type 1C

Mucocutaneous Bleeding
High Index of Suspicion for VWD

- VWD Standard Testing
  - VWF:AG, VWF:RCo, FVIII:C Platelet Count

- VWF:RCo/VWF:Ag < 0.6

Disorders of VWF Binding to platelets

- Multimers Normal
  - Type 2M

- Loss of High Molecular Weight Multimers

- RIPA Enhanced
  - VWD Type 2B

- RIPA Normal
  - VWD Type 2A

Disorder of VWF Binding to FVIII

- FVIII Disproportionately Low compared to VWF:Ag

- Abnormal VWF:FVIII Binding Capacity
  - VWD Type 2N

BLOOD, 26 MARCH 2015 x VOLUME 125, NUMBER 13: Ng, C, Motto, DG, Di Paola, J
Conclusion

• VWD is a **heterogeneous** group of clinical manifestations

• Requires a **battery of tests** for proper diagnosis
  - New vWF:Activity assays are emerging
    • Replacing ristocetin and platelets with latex-coated beads
  - VWF:CB is becoming part of the routine test panel when screening for absence of HMWM’s
  - Supplemental tests are useful in the evaluation of the qualitative defects in VWD

• *Distinction between “low-vWF” and Type 1 vWD is still being debated*
Conclusion

LABORATORY DIAGNOSIS OF VON WILLEBRAND DISEASE

LARRY J. SMITH

ABSTRACT
Von Willebrand disease (VWD) is considered the most common congenital bleeding abnormality in the world and an accurate diagnosis is often very challenging.

INTRODUCTION
Accurate diagnosis of von Willebrand disease (VWD) can often be very challenging for clinicians. In addition to patient and family history, clinicians utilize a panel of


Larry J Smith, PhD, SH(ASCP), Abbott Diagnostics Division – Hematology Business Unit, Santa Clara, CA

Address for Correspondence: Larry J Smith, PhD, SH(ASCP), Abbott Diagnostics Division – Hematology Business Unit, 4551 Great America Pkwy, Santa Clara, CA 95054
QUESTIONS???

Thank you!