von Willebrand Disease

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Objectives

• Define von Willebrand Disease
• Describe current classification of vWD
• Review structure and function of vWF
• Discuss methods for diagnosing vWD and limitations
• Appreciate the influence of preanalytical variables influencing vWD testing

• Disclosure
  ▫ Consultation: Instrumentation Laboratory
Case 1

- 18 y-o-f presents with easy bruising, heavy menses, prolonged bleeding after wisdom tooth extraction

- Lab values
  - PT = 12.0 sec  \( (9.5 – 12.8 \text{ sec}) \)
  - aPTT = 38.0 sec  \( (24.0 – 36.3 \text{ sec}) \)
  - FVIII = 45%  \( (50 – 150\%) \)
  - FIX = 105 %  \( (50 – 150\%) \)
  - FXI = 113%  \( (50 – 150\%) \)
  - vWF:ACT = 48%  \( (50 – 150\%) \)
  - vWF:Ag = 52%  \( (50 – 150\%) \)
Case 2

• 32 y-o-f presents with 2\textsuperscript{nd} trimester pregnancy. She has had excessive bleeding after arthroscopy, and a history of a brother who died from bleeding at 12 years of age after an appendectomy. No history of excessive bruising.

• Lab values
  ▫ PT = 11.5 sec (9.5 – 12.8 sec)
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  ▫ FVIII = 5% (50 – 150%)
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• 55 y-o-f, hematology consult for hemorrhage after hysterectomy for cervical cancer. Life-long history severe bleeding with minimal trauma. At 13, with first menses, she had massive hemorrhage and received “radioactive cobalt” implants in her ovaries to stop bleeding. Brother died of hemorrhage at age 2.

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Case 4

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

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  - PT = 12.2 sec \((9.5 – 12.8 \text{ sec})\)
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Case 5

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

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  - vWF:Ag = 51%  (50 – 150%)
  - PLT CT = 1.250 x 10^9/L
vWD

- Autosomally inherited bleeding disorder that is caused by deficiency or dysfunction of vWF
  - The Diagnosis, Evaluation, and Management of von Willebrand Disease, 2007, NIH publication 08-5832
vWD—Disorder of Primary Hemostasis

- Most common of the congenital bleeding disorders
  - 1-2 % of the general population
  - Symptomatic in only about 1/10,000
- 1926 – Erik von Willebrand → 5 y-o-f and her family who lived on the Åland Islands – Hereditär pseudohemofili, 1926
- Initially described as “pseudohemophilia”
vWD—Disorder of Primary Hemostasis

- Clinical manifestations
  - Mucocutaneous bleeding of varying severity in males and females
    1. Ecchymoses
    2. Epistaxis
    3. Gastrointestinal bleeding
    4. Menorrhagia
  - Defective platelet adhesion
  - Reduced FVIII levels
**vWF**

- Large multimeric protein – ranges from 600 kD to >20 million kD
  - Synthesized by endothelial cells and megakaryocytes
    - Endothelial cells source of plasma vWF
- Gene for vWF is located on chromosome 12p
  - 178 kB, 52 exons

Synthesis of vWF

- vWF synthesized in **endothelial cells** and **megakaryocytes**
  1. Stored in **Weibel-Palade** bodies of endothelial cells
  2. Stored in **α-granules** of platelets

Steps in synthesis of vWF

1. First synthesized as a **pre-pro-vWF monomer**
2. **Dimerization** occurs in **ER**
3. Pre-pro-vWF monomers **linked together** at the carboxyl terminal end
4. Dimeric molecules pass to the **Golgi apparatus**
5. Dimers **multimerize**
6. Propeptide is **cleaved off** → **mature** subunit
vWF Release


Normal Subject

Cleaved unusually large multimers of von Willebrand factor

ADAMTS 13

Binding site

Endothelial cell

Secretion of multimers from Weibel-Palade body
Function of vWF

- vWF serves two important biologic functions
  1. Serves as a **carrier protein** for plasma FVIII
     a. VWF **protects Factor VIII** in circulation
     b. VWF **co-localizes FVIII** at sites of vascular injury
  2. Serves as a ligand that binds to the gpIb receptor on platelets to initiate platelet **adhesion** to the damaged endothelium
     a. VWF binds to **extravascular collagen**
     b. Platelets adhere to the bound vWF
     c. Adherent platelets become activated
Function of vWF
B. PRIMARY HEMOSTASIS

1. Platelet adhesion
2. Shape change
3. Granule release (ADP, TXA₂)
4. Recruitment
5. Aggregation (hemostatic plug)
Classification of vWD

- vWD – extremely heterogeneous, complex disorder with > 20 distinct subtypes

- Types of vWD
  1. **Quantitative Defects**
     - **Type 1**
       - Partial quantitative deficiency
       - Autosomal dominant
     - **Type 3**
       - Complete absence/severely decreased
       - Autosomal recessive
  2. **Qualitative Defects**
     - **Type 2**
       - 2A
       - 2B
       - 2M
       - 2N
       - Autosomal recessive

Subgroups
Quantitative Defects
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

- Endothelial cells and platelets contain **normal**, but **reduced** levels of vWF
  - DDAVP can induce the release the **stored** vWF

- Bleeding symptoms range from **asymptomatic to mild**
Type I vWD

- Lab findings
- Normal to decreased
  1. FVIII (aPTT)
  2. vWF:Activity (Ristocetin Cofactor)
  3. vWF:Antigen

4. Prolonged BT
   • (PFA-100—Col/EPI, Col/ADP)
5. Proportional decrease of ALL vWF multimers
Type 3 vWD

- **Most severe** form of the disease

- Results from the **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
Type 2A vWD

- Mutations commonly occur in the A2 region
- Presence of only the smaller vWF multimers in plasma → reduced binding to platelets
  - **LOSS** platelet-dependent function

- **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
- Absence of the high-molecular-weight multimers
  - Caused by “**gain of function**” mutation in vWF → increased affinity to bind to the gpIb platelet receptor
  - **Spontaneous binding** of vWF to platelets
  - Large multimers are synthesized but rapidly cleared due to increased binding to platelets
  - **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

- Normal multimer profile

- Plasma binding to FVIII is normal
Type 2N vWD

- Also referred to as “autosomal hemophilia” or the Normandy variant
- Caused by mutations in the FVIII binding region of vWF

- Markedly decreased affinity for binding to FVIII
  - Rapid turnover of the unbound FVIII → reduced levels

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

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

- **NO** genetic defect of the vWF molecule – vWF molecule is NORMAL

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

- Defect is in the platelet → standard approaches to treating vWD are not helpful

- **Lab findings**
  1. Loss of high molecular weight multimers
  2. **Platelet count is low**
  3. Platelet aggregation with low dose ristocetin (RIPA)
Acquired vWD

- Qualitative, structural, or functional disorder of vWF not inherited and is associated with an increased risk of bleeding
- Associated with
  - Autoimmune clearance – lymphoproliferative, MGUS, SLE, hypothyroidism
    - Autoantibodies \(\rightarrow\) increased clearance of vWF from plasma
  - Fluid shear stress-induced proteolysis – aortic stenosis, LVAD

<table>
<thead>
<tr>
<th>Aspect</th>
<th>Acquired vWD</th>
<th>Congenital vWD</th>
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<tbody>
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<td>Personal History</td>
<td>Late onset bleeding</td>
<td>Early onset bleeding</td>
</tr>
<tr>
<td>Family History</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>AVWS associated disorder</td>
<td>Positive</td>
<td>Negative</td>
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<tr>
<td>Laboratory associated disorder</td>
<td>Inhibitor to vWF</td>
<td>Genetic mutation</td>
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<tr>
<td>Treatment</td>
<td>• Remission after IVIg</td>
<td>vWF-containing product</td>
</tr>
<tr>
<td></td>
<td>• Short lived response after vWF-containing product</td>
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</table>
Assays for vWD

- Platelet Function Screen (BT)
- vWF antigen assay
- vWF activity assay
- FVIII:C

- Multimer Analysis
Assays for vWD

PFA-100 (Bleeding time)
Assays for vWD

- **vWF:Antigen**
  - Immunoassay that measures the concentration of vWF protein in plasma
    - Actual protein responsible for binding to FVIII and gp Ib/IX/V complex
    - Detects all forms of vWF (functional and nonfunctional forms)
    - Cannot discriminate between multimer size

**LIA based testing**

Instrument reading—changes in optical density secondary to aggregates
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
Assays for vWD

- **vWF:Activity**
  - Latex particle enhanced immunoturbidimetric assay
    - *Specific anti-vWF monoclonal antibody* adsorbed onto latex reagent directed against the platelet binding site of vWF (gp Ib receptor)
    - Reacts with vWF in the patient’s plasma
    - Degree of agglutination is directly proportional to activity of vWF in patient's plasma
      - *Mix patient’s plasma + latex beads coated with an anti-vWF monoclonal antibody ➔ agglutination of particles*
Assays for vWD

- **FVIII**
  - Circulating level of FVIII
  - Clot-based assay that measures the ability of plasma FVIII to shorten the clotting time in FVIII-deficient plasma

- **Multimer Analysis**
  - *Qualitative assay (electrophoresis) to depict the variable concentrations of different-sized vWF multimer*
Additional Assays

Ristocetin Induced Platelet Aggregation

- 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

Collagen Binding Assay

- Measures the ability of large vWF multimers to bind collagen
- vWF binds collagen via A3 and A1 domains
- 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
Additional Assays

• vWF Platelet Binding Assay
  ▫ Measures the ability of patient vWF to bind to normal formalin-fixed platelets in the presence of a low concentration of ristocetin
  ▫ Differentiate Platelet-type vWD from Type 2B vWD

• vWF FVIII-Binding Assay
  ▫ Measures the ability of patient vWF to bind to recombinant FVIII
  ▫ Type 2N has decreased binding to FVIII
Preanalytical Variables

- 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
Preanalytical Variables

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

- **Age**
  - Levels increase with age

- **ABO Blood Groups**

<table>
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<tr>
<th>ABO Type</th>
<th>N</th>
<th>Mean</th>
<th>Mean +/- 2SD</th>
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<tr>
<td>O</td>
<td>456</td>
<td>74.8</td>
<td>35.6 – 157.0</td>
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<tr>
<td>A</td>
<td>240</td>
<td>105.9</td>
<td>48.0 – 233.9</td>
</tr>
<tr>
<td>B</td>
<td>196</td>
<td>116.9</td>
<td>56.8 – 241.0</td>
</tr>
<tr>
<td>AB</td>
<td>109</td>
<td>123.3</td>
<td>63.8 – 238.2</td>
</tr>
</tbody>
</table>
Preanalytical 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
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 be given as an inhaler—Stimate
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 and VWF
   ▫ Only plasma fraction that consistently contains VWF multimers
   ▫ **USE WITH CAUTION!!!**
## 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>
<th>Type 2N</th>
<th>Type 3</th>
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<tbody>
<tr>
<td>PFS</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>N</td>
<td>↑↑↑</td>
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<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>
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<tr>
<td>vWF:AG</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓ or (N)</td>
<td>↓ or (N)</td>
<td>↓↓↓</td>
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<td>RCOFTR</td>
<td>↓</td>
<td>↓↓↓</td>
<td>↓↓↓</td>
<td>↓↓↓</td>
<td>↓ or (N)</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>RIPA</td>
<td>None</td>
<td>None</td>
<td>↑</td>
<td>↑</td>
<td>None</td>
<td>None</td>
<td>None</td>
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<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>
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**Levels of Lab Test Values:**
- **High**
- **Intermed**
- **Low**
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Conclusion

- vWD is a heterogeneous group of clinical manifestations
- Requires a battery of tests for proper diagnosis
- Type 1 is a complex quantitative defect
  - Distinction between “low-vWF” and Type 1 vWD is still being debated
- New vWF:Activity assays are emerging
  - Replacing ristocetin and platelets with latex-coated beads
- vWF:CB may become 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
Questions???

Thank you!