von Willebrand Disease
Hemostasis Laboratory Update

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## Disclosures for Ken Friedman, M.D.

<table>
<thead>
<tr>
<th>Role</th>
<th>Information</th>
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<tbody>
<tr>
<td>Research Support/P.I.</td>
<td>No relevant conflicts of interest to declare</td>
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<tr>
<td>Employee</td>
<td>BloodCenter of Wisconsin</td>
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<tr>
<td>Consultant</td>
<td>Novo Nordisk, CSL Behring, Baxalta</td>
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<td>Major Stockholder</td>
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<td>Speakers Bureau</td>
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<td>Honoraria</td>
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<td>Scientific Advisory Board</td>
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This presentation includes no discussion of off-label use of a drug or medical device:
Outline

• Background
  – VWF functions
  – VWD disease classification

• Update on functional assays of VWF
  – Interaction of VWF with platelets
  – Interaction of VWF with collagen
  – Does VWF survive in circulation: VWF propeptide antigen

• Summary statements
von Willebrand Factor (VWF)

- VWF has 3 main functions
  - Chaperone of Factor VIII
  - Binding to exposed subendothelial collagen
  - Capture of platelets at sites of vascular injury

- von Willebrand disease (VWD)
  - Bleeding disorder attributable to inadequate VWF function
Classification of von Willebrand Disease

- **Inherited Quantitative Defects:**
  - Type 1: Low VWF protein level
  - Type 3: Absence of VWF

- **Inherited Qualitative Defects:**
  - Type 2
    - Poor platelet-binding function:
      - Due to small multimers: Type 2A
      - Normal size multimers (abnormal A1 loop): Type 2M
    - Poor collagen-binding function: Type 2M
    - Accentuated platelet-binding function:
      - Abnormal A1 loop of VWF: Type 2B
      - Abnormal platelet GP Ib: Platelet-type
      - Decreased Factor VIII Binding: Type 2N

- **Acquired VWD**
Initial Evaluation for VWD
VWD Guideline – NHLBI Expert Panel

• No single lab screening test for VWD

• Quantitative assays:
  • VWF Ristocetin cofactor activity
    – Functional ability of VWF to bind and agglutinate platelets in the presence of ristocetin
  • VWF antigen
    – Immunoassay of VWF protein present
  • Factor VIII activity
    – Coagulant activity of Factor VIII

• Ratio: VWF:RCo/VWF:Ag and FVIII/VWF:Ag
Additional Testing for VWD

- Additional tests depend on initial results
  - Multimer distribution
  - VWF collagen binding (VWF:CB)
  - VWF propeptide (VWFpp)
  - VWF:FVIII binding assay
  - Ristocetin-induced platelet aggregation
  - VWF gene sequencing
Update on Functional Assays of VWF

• Does VWF Function?
  – Platelet Binding Function
  – Collagen Binding Function

• Does VWF survive in circulation?
  – VWF propeptide antigen
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<td>Ristocetin cofactor activity: “traditional” assay uses ristocetin to induce VWF binding to platelets</td>
<td><img src="image" alt="Diagram" /> Platelet + ristocetin + VWF</td>
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### Advantages

- **“Historic Standard”** for measuring VWF activity
- Most data correlating VWF levels and desmopressin/replacement treatment reported VWF:RCo
- Fully automated VWF:RCo assays widely available

### Disadvantages

- Poor sensitivity (LOD ≥ 10 IU/dL)
  - Difficult to characterize patients with severe VWD
- VWF:RCo/VWF:Ag ratio is critical for subclassification; high CV may lead to false diagnoses in moderately severe VWD
- Not the physiologic activator of VWF activity
  - VWF variant p.D1472H causes spuriously decreased VWF:RCo (assay artifact)

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**Platelet + ristocetin + VWF**


Chart courtesy of Sandra Haberichter
Interaction of VWF With Platelets
Polymorphisms interfere with ristocetin binding

How common is D1472H?
- African Americans: 63% D1472H+
- Caucasians: 19% D1472H+

Figure 7. VWF:RCo assay compared with VWF GPIb complex-binding assay. The first 2 columns show VWF:RCo/VWF:Ag ratio (●) for subjects with and without the D1472H polymorphism. 1

## VWF Platelet-binding Activity: Nomenclature

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<td>VWF:GPIbR</td>
<td>Reports ristocetin-induced binding of VWF to recombinant wild-type GPIb fragment</td>
<td><img src="image" alt="OR" /> rWT-GPIb + ristocetin + VWF</td>
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Courtesy of Sandra Haberichter
VWF:GPIbR – Pros and Cons

**Advantages**

- Automated assay applications are precise (CV ~ 6-8%) and sensitive (LOD to 0.5 - 5 IU/dL)
  - Available in some countries as latex or magnetic particle-enhanced automated assays
- Correlation with VWF:RCo reported to be excellent

**Disadvantages**

- Not a physiologic activator of VWF activity
  - Ristocetin allows spuriously decreased activity for VWF p.D1472H variant
- Not commercially available in USA

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<td>VWF:GPIbM</td>
<td>Reports spontaneous binding of VWF to gain-of-function mutant GPIb fragment</td>
<td><img src="image3.png" alt="Gain-of-function rGPIb + VWF" /></td>
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*Courtesy of Sandra Haberichter*
### VWF:GPIbM – Pros and Cons

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<th>Advantages</th>
<th>Disadvantages</th>
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<tr>
<td>➢ Gain of function GPIb allow for spontaneous binding of VWF</td>
<td>➢ Automated application may not discriminate between types 2A and 2B VWD</td>
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<tr>
<td>– Correlates well with VWF:RCo</td>
<td>➢ Reports slightly lower that VWF:RCo → ? More type 2 cases</td>
</tr>
<tr>
<td>– Not subject to false low values when p.D1472H present</td>
<td>➢ Heterophile antibody may interfere with assay</td>
</tr>
<tr>
<td>– ELISA method may discriminate between types 2A and 2B VWD</td>
<td>➢ Not commercially available in USA</td>
</tr>
<tr>
<td>➢ Automated applications of assay are precise (CV ~ 7%) and sensitive (LOD ~2 - 6 IU/dL)</td>
<td>OR</td>
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## VWF Platelet-binding Activity: Nomenclature

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<td>VWF:Ab</td>
<td>Reports binding of a monoclonal antibody to a VWF A1 domain epitope</td>
<td>![Diagram](Anti-A1 MoAb + VWF)</td>
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Courtesy of Sandra Haberichter
### Advantages

- LIA version performed better than ELISA in discriminating subtypes
- User-friendly, applicable to several platforms, feasible for routine laboratories
- Generally good correlation with VWF:RCo

### Disadvantages

- Not truly a VWF functional assay
- Some VWD type 2M mutations (p.G1324A) are not detected
- No improvement in LOD (19 IU/dL) compared to VWF:RCo
- Screening of VWF patients best when combined with other tests
  - May miss some type 2
- Not recommended by ISTH as a replacement for VWF:RCo

*Anti-A1 MoAb + VWF*


Chart courtesy of Sandra Haberichter
VWF Platelet-Binding Function Assay Summary

• All the automated techniques allow with higher precision

• Recent commercial methods are replacing platelets with recombinant GPIb fragments (ELISA or latex/magnetic bead-based)

• Newer assays have improved sensitivity and precision, allowing accurate measurement of very low VWF levels (<1 – 6 IU/dL)

• Ristocetin-free assays prevent problems with false-positive results in certain populations

“For these reasons, the time-honored notion that VWF:RCo is indispensable is rapidly changing”¹

Appropriate nomenclature should be used in lab reports

– Although the lab report may state “VWF Activity”, they don’t all measure the interaction of VWF with GP-Ib

– Ristocetin-based assays of VWF platelet-binding function measure both ristocetin binding as well as platelet binding, and reported result may be low if D1472 is present
Update on Functional Assays of VWF

- **Does VWF Function?**
  - Platelet Binding Function
  - Collagen Binding Function

- **Does VWF survive in circulation?**
  - VWF propeptide antigen
Quantitative assay based upon preferential binding of larger VWF multimers to collagen
  • More sensitive than VWF:RCo to loss of highest multimers
VWF binds collagen via A3 and A1 domains
  • Type 2M VWD may be due to a collagen binding defect

Assay characteristics
  • ELISA format
  • Low limit of detection is similar to VWF:Ag
  • Has lower CV than VWF:RCo
VWF Collagen-Binding Activity
A Sensitive Screen of Multimer distribution

- VWF:CB/VWF:Ag ratio correlated with the multimer distribution\textsuperscript{1}
  - VWF:CB is a highly sensitive screen for a defect of VWF multimer distribution\textsuperscript{1-3}

\textsuperscript{1} Flood, V.H., et al. *Clinical Chemistry*; 2013; 59: 684-691
\textsuperscript{2} Adcock D. *Sem Thromb Hemost* 2006; 32:472-476.2007
\textsuperscript{3} Favaloro EJ. *Sem Thromb Hemost* 2009; 35: 60-75
VWF Function: Collagen Binding

- VWF A3 domain binds collagens 1 and 3  
  Pareti et al, J Biol Chem 1987
- VWF A1 domain binds collagens 4 and 6  
  Rand et al, J Clin Invest 1991
Defective collagen binding in subset of VWD patients in Zimmerman Study

<table>
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<tr>
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<th>Healthy controls</th>
<th>Type 1 VWD</th>
<th>Type 2M VWD</th>
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<tbody>
<tr>
<td>VWF:CB/VWF:Ag</td>
<td>1.06</td>
<td>0.95</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.87</td>
<td>1.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.65</td>
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Collagen 3
Collagen 4

Courtesy of VH Flood
Increased Bleeding in Type 2M VWD With Decrease in VWF:CB4/VWF:Ag

Bleeding Score (ISTH BAT)

Low VWF:CB4/Ag Ratio < 0.7

Normal VWF:CB4/Ag Ratio > 0.7

10.5 Median 6

Courtesy of VH Flood
VWF Collagen-Binding Function Defects found in VWD Patients

- **VWF A3 Domain:** Binds types 1 and 3 collagen
  - At least 5 mutations reported in VWD
  - Rare (<1%) in Zimmerman Program participants

- **VWF A1 Domain:** Binds types 4 and 6 collagen
  - At least 7 mutations reported
  - Relatively common in Zimmerman Program participants
    - 6% of type 1 subjects
    - 19% of type 2M subjects

VWF Collagen-Binding Activity Summary

• VWF:CB/VWF:Ag ratio is highly sensitive to multimer structure and may be used to predict multimer defects

• Collagen binding assays evaluate a discrete VWF function
  – Collagens 1 and 3 bind in VWF A3 domain
  – Collagens 4 and 6 bind in VWF A1 domain,
    – Collagen 4 and 6 assays are not yet clinically available

• Defects of collagen binding occur in VWD
  – Collagen binding defects may contribute to bleeding risk
  – We don’t find these defects if we don’t look for them
Update on Functional Assays of VWF

• Does VWF Function?
  – Platelet Binding Function
  – Collagen Binding Function

• Does VWF survive in circulation?
  – VWF propeptide antigen
Type 1C VWD
Due to Increased VWF Clearance

• Mechanism
  – Short half-life of VWF\(^1\)
• Laboratory findings are subtly different from type 1 VWD
  – Factor VIII is reduced to a similar extent as VWF:Ag
  – Larger than normal multimers may be observed\(^2\)
  – Initial “hyper-fold” rise of VWF in response to desmopressin
    • But VWF elevation not sustained, half-life only 1-4 hours
      – VWF replacement provides more sustained VWF levels
• Genetics
  – Autosomal dominant: Mutations in D3, A1 and D4 domains

VWF propeptide (VWFpp) Utility in VWD with Increased Clearance

- VWF propeptide (VWFpp)
  - Directs VWF multimerization and storage in cells
  - Is co-released with mature VWF from endothelial cells
- By definition, 1 mL of normal pooled plasma contains:
  - 1 unit of VWF:Ag and 1 unit of VWFpp (VWFpp/VWF:Ag ~ 1)
  - Increased VWFpp/VWF:Ag is a marker of VWF clearance
  - Diagnostically useful in:
    - Type 1C/Vicenza
    - Acquired VWD

Type 1C Comprises the Majority of Severe Type 1 VWD Cases
Data from Zimmerman Study

- Percentage of quadrant consistent with type 1C phenotype: 76%, 38%, 7%

Graph showing VWF:Ag (IU/dL) vs. VWFpp/VWF:Ag with lines indicating reduced secretion and increased clearance.
Screening studies are subtly different between Type 1C and type 1 VWD
  - Type 1C VWD compromises a substantial proportion of the cases of Type 1 VWD when the VWF antigen is under 20 IU/dL

VWFpp/VWF:Ag ratio is a marker of VWF clearance
  - VWFpp is a useful marker to identify type 1C and acquired VWD
    • Note: Ratio may also abnormal in types 2A and 2B
Conclusions

• Classification of VWD remains complex and utilization of new VWF assays may aid in proper VWD diagnosis, providing direction for optimal treatment strategies.

• “VWF platelet-binding activity” assays include several assays which may measure ristocetin binding in addition to platelet binding, or alternatively, binding of VWF to an A1 domain specific antibody.

• Collagen binding assays evaluate a discrete function of VWF and isolated collagen binding defects may contribute to bleeding symptoms.

• Assay of VWFpp can identify Type 1C VWD, a common condition among type 1 patients with VWF:Ag <20.