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• This speaker has nothing to disclose.
Laboratory Diagnosis of Von Willebrand Disease

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Pathology Resident
University of Utah
Objectives

After attending this seminar, the attendee are expected to be able to:

• Explain common symptoms and genetic causes of von Willebrand disease.

• Describe the common laboratory assays used to diagnose von Willebrand disease.

• List common subtypes of von Willebrand disease.
Topics

1. Introduction of von Willebrand factor and von Willebrand disease

2. Common diagnostic tests for von Willebrand disease and interpretation

3. Case studies
1. Introduction of von Willebrand factor and von Willebrand disease

2. Common diagnostic tests for von Willebrand disease and interpretation

3. Case studies
Von Willebrand factor (vWF)

vWF is synthesized in endothelial cells as a monomer that is subsequently made into multimers that are secreted.

vWF is a critical protein in blood clotting.
Von Willebrand factor (vWF)

The majority of vWF is circulating in the blood plasma.

A pool of vWF is also stored in the endothelial cells and megakaryocytes, the precursors of platelets.
Von Willebrand factor (vWF)

Propeptide

Mature vWF

domains:

23 aa 763 764

signal peptide aa 1-22

2813

FVIII  GPIb  Collagen  GPIIb/IIIa

FVIII: Factor VIII

GPIb: platelet glycoprotein Ib

GPIIb/IIIa: platelet glycoprotein IIb/IIIa

vWD Guidelines, NHLBI
Von Willebrand factor (vWF)

vWF is a multimeric protein composed of dimeric building blocks.

Body Reactions to Bleeding

1. Constriction of blood vessels

Robbins and Cotran Pathologic Basis of Disease 8th edition
Body Reactions to Bleeding

2. Adhesion of platelets

Robbins and Cotran Pathologic Basis of Disease 8th edition
3. Formation of fibrin reinforces platelets
vWF Plays Two Major Roles

- vWF tethers the platelet to exposed collagen
- vWF serves as a carrier protein for factor VIII

Kumar: Robbins Basic Pathology, 9th ed.
Von Willebrand disease (vWD) was first described in 1926 by a Finnish physician named Dr. Erik von Willebrand.

Quantitative deficiency of vWF or to functional deficiencies of vWF

Autosomal inheritance pattern / Males and females are affected equally
Von Willebrand disease (vWD)

The first manuscript describing a haemorrhagic disorder in people who were living on the Aland islands off the coast of Finland.
Von Willebrand disease (vWD)

His first case was a little girl, who was five years old when first examined.

She was one of 12 siblings, all but two of whom had had bleeding symptoms.

Her parents had severe nose bleedings.

The girl herself had had several severe episodes of bleeding from the nose and lips, and following tooth extractions. At the age of 13, she bled to death during her fourth menstrual period.
Von Willebrand disease (vWD)

Most frequent inherited bleeding disorder

Estimated prevalence of 1% in general population

Clinically significant vWD: 100 persons per million population
Clinical Manifestations

- Nosebleed
- Trauma/wounds
- Easy bruising
- Dental extractions
- Gingival bleeding
- Heavy or prolonged menstrual period
- Digestive tract bleeding
<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
<th>Disease Severity</th>
<th>Percentage of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>Partial quantitative deficiency of vWF</td>
<td>Mild-moderate disease</td>
<td>70%</td>
</tr>
<tr>
<td>Type 2</td>
<td>Qualitative deficiency of vWF</td>
<td>Mild to moderate disease</td>
<td>25%</td>
</tr>
<tr>
<td>Type 3</td>
<td>Total or near total quantitative deficiency of vWF</td>
<td>Severe disease</td>
<td>5%</td>
</tr>
</tbody>
</table>
**vWD Classification**

<table>
<thead>
<tr>
<th>Type 1: Partial quantitative deficiency of vWF</th>
<th>Mild-moderate disease</th>
<th>70%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 2: Qualitative deficiency of vWF</td>
<td>Mild to moderate disease</td>
<td>25%</td>
</tr>
<tr>
<td>Type 3: Total or near total quantitative deficiency of vWF</td>
<td>Severe disease</td>
<td>5%</td>
</tr>
</tbody>
</table>
vWD Classification

<table>
<thead>
<tr>
<th>Type 2A</th>
<th>↓vWF-dependent platelet adhesion with selective deficiency of high molecular weight vWF multimers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 2B</td>
<td>Increased vWF affinity for platelet GPIb; ± ↓platelet numbers</td>
</tr>
<tr>
<td>Type 2M</td>
<td>↓vWF-dependent platelet adhesion without selective deficiency of high molecular weight vWF multimers</td>
</tr>
<tr>
<td>Type 2N</td>
<td>Markedly decreased vWF binding affinity for FVIII</td>
</tr>
</tbody>
</table>
Topics

1. Introduction of von Willebrand factor and von Willebrand disease

2. Common diagnostic tests for von Willebrand disease and interpretation

3. Case studies
Initial vWD testing

- von Willebrand Factor Antigen
- von Willebrand Factor Activity (Ristocetin Cofactor)
- Factor VIII Activity
von Willebrand Factor Antigen

vWF:Ag

Immunological assay that measures the concentration of the vWF protein in plasma.

Methodology: Microlatex Particle-Mediated Immunoassay
Principle of Latex Immunoassay

Ab-coated Latex Particles | Patient Plasma Added | Ag-Ab Binding and Precipitation

Turbidity ↑
Absorbance ↑

http://tiger.kobiljak.msu.edu/WebSites/Web_Path/webpath/microbio/microbe/serology.htm
von Willebrand Factor Activity (Ristocetin Cofactor)

- vWF:RCo
- Measures the ability of a patient’s plasma to agglutinate platelets in the presence of the antibiotic Ristocetin.
- Methodology: Platelet Agglutination
von Willebrand Factor Activity (Ristocetin Cofactor)
von Willebrand Factor Activity (Ristocetin Cofactor)

- Ristocetin is an antibiotic
- Side effect: activates vWF and induces platelet agglutination and cause thrombocytopenia
- Removed from the market
Principles of von Willebrand Factor Activity Assay

Platelets → Ristocetin → vWF → Platelet Agglutination
In quantitative vWF deficiency (types 1 and 3), it parallels the vWF antigen.

In qualitative vWF deficiency resulting in decreased affinity for platelets (types 2A and 2M), vWF RCo is more severely affected compared to vWF antigen.
However this assay is not a true 'functional' assay but rather the interaction of vWF with the Gp1b platelet receptor in the presence of ristocetin.
Factor VIII Activity

Measures the activity of factor VIII

Functional clot-based assay
## Result Interpretation

Type 1: **Partial quantitative** deficiency of vWF

<table>
<thead>
<tr>
<th>Condition</th>
<th>vWF:RCo (%)</th>
<th>vWF:Ag (%)</th>
<th>FVIII (%)</th>
<th>Ratio of vWF:RCo/vWF:Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>51-215</td>
<td>52-214</td>
<td>56-191</td>
<td>&gt;0.5-0.7</td>
</tr>
<tr>
<td>Type 1</td>
<td>&lt;30</td>
<td>&lt;30</td>
<td>↓ or Normal</td>
<td>&gt;0.5-0.7</td>
</tr>
</tbody>
</table>
## Result Interpretation

Type 3: **Total quantitative** deficiency of vWF

<table>
<thead>
<tr>
<th>Condition</th>
<th>vWF:RCo (%)</th>
<th>vWF:Ag (%)</th>
<th>FVIII (%)</th>
<th>Ratio of vWF:RCo/vWF:Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>51-215</td>
<td>52-214</td>
<td>56-191</td>
<td>&gt;0.5-0.7</td>
</tr>
<tr>
<td>Type 3</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>↓ ↓ ↓</td>
<td>N/A</td>
</tr>
</tbody>
</table>
### Result Interpretation

**Type 2A**: ↓vWF-dependent platelet adhesion with selective deficiency of high molecular weight vWF multimers

<table>
<thead>
<tr>
<th>Condition</th>
<th>vWF:RCo (%)</th>
<th>vWF:Ag (%)</th>
<th>FVIII (%)</th>
<th>Ratio of vWF:RCo/vWF:Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>51-215</td>
<td>52-214</td>
<td>56-191</td>
<td>&gt;0.5-0.7</td>
</tr>
<tr>
<td>Type 2A</td>
<td>&lt;30</td>
<td>30-200</td>
<td>↓ or Normal</td>
<td>&lt;0.5-0.7</td>
</tr>
</tbody>
</table>
Result Interpretation

Type 2B: Increased vWF affinity for platelet GPIb; ±↓platelet numbers

<table>
<thead>
<tr>
<th>Condition</th>
<th>vWF:RCo (%)</th>
<th>vWF:Ag (%)</th>
<th>FVIII (%)</th>
<th>Ratio of vWF:RCo/vWF:Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>51-215</td>
<td>52-214</td>
<td>56-191</td>
<td>&gt;0.5-0.7</td>
</tr>
<tr>
<td>Type 2B</td>
<td>&lt;30</td>
<td>30-200</td>
<td>↓ or Normal</td>
<td>Usually &lt;0.5-0.7</td>
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</tbody>
</table>

Ristocetin-Induced Platelet Aggregation (RIPA)

Type 2B: Increased platelet aggregation at low dose of ristocetin
Result Interpretation

Type 2M: ↓vWF-dependent platelet adhesion without selective deficiency of high molecular weight vWF multimers

<table>
<thead>
<tr>
<th>Condition</th>
<th>vWF:RCo (%)</th>
<th>vWF:Ag (%)</th>
<th>FVIII (%)</th>
<th>Ratio of vWF:RCo/vWF:Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>51-215</td>
<td>52-214</td>
<td>56-191</td>
<td>&gt;0.5-0.7</td>
</tr>
<tr>
<td>Type 2M</td>
<td>&lt;30</td>
<td>30-200</td>
<td>↓ or Normal</td>
<td>&lt;0.5-0.7</td>
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</tbody>
</table>
### Result Interpretation

**Type 2N**: Markedly decreased vWF binding affinity for FVIII

<table>
<thead>
<tr>
<th>Condition</th>
<th>vWF:RCo (%)</th>
<th>vWF:Ag (%)</th>
<th>FVIII (%)</th>
<th>Ratio of vWF:RCo/vWF:Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>51-215</td>
<td>52-214</td>
<td>56-191</td>
<td>&gt;0.5-0.7</td>
</tr>
<tr>
<td>Type 2N</td>
<td>30-200</td>
<td>30-200</td>
<td>↓ ↓</td>
<td>&gt;0.5-0.7</td>
</tr>
</tbody>
</table>

- vWF:FVIII binding (vWF:FVIIIIB) assay
von Willebrand Factor Multimers

vWF monomer is about 250 kD

vWF is released from endothelial cells to the plasma as a multimers ranging from 500-20,000 kD

Analysis of vWF multimeric forms by this procedure is predominantly designed to evaluate type II vWD
Principle of Gel Electrophoresis and Western Blot
Principle of Gel Electrophoresis and Western Blot

http://www.nhlbi.nih.gov/guidelines/vwd/3_diagnosisandevaluation.htm
Principle of Gel Electrophoresis and Western Blot

<table>
<thead>
<tr>
<th>vWD Type 1</th>
<th>NP</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP</td>
<td>vWD Type 2A</td>
</tr>
</tbody>
</table>
Genetic testing

Type-specific sequencing tests
## Genetic testing

<table>
<thead>
<tr>
<th>Description</th>
<th>Code</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>von Willebrand Disease, Type 2A (<em>VWF</em>) Sequencing Exon 28 with Reflex to 9 Exons</td>
<td>2005480</td>
<td>Polymerase Chain Reaction/Sequencing</td>
</tr>
<tr>
<td>von Willebrand Disease, Type 2B (<em>VWF</em>) Sequencing</td>
<td>2005486</td>
<td>Polymerase Chain Reaction/Sequencing</td>
</tr>
<tr>
<td>von Willebrand Disease, Type 2M (<em>VWF</em>) Sequencing</td>
<td>2005490</td>
<td>Polymerase Chain Reaction/Sequencing</td>
</tr>
<tr>
<td>von Willebrand Disease, Type 2N (<em>VWF</em>) Sequencing</td>
<td>2005494</td>
<td>Polymerase Chain Reaction/Sequencing</td>
</tr>
</tbody>
</table>
Topics

1. Introduction of von Willebrand factor and von Willebrand disease

2. Common diagnostic tests for von Willebrand disease and interpretation

3. Case studies
Case 1

A one-year-old girl was referred to our hospital for prolonged oral bleeding following a mouth wound. Physical examination revealed many bruises. There was no documented familial history of hemorrhage.
Case 1

Lab Results:

Platelet count: 346 (229-465 K/ul)

aPTT (Partial Thromboplastin Time): 68 s (24-35 s)

vWF:Ag <10% (0-6 years: 52-214%)
vWF:RCo <10% (0-6 years: 51-215%)
Factor VIII 7% (0-6 years: 56-191%)
Case 1

What is the most likely diagnosis:
A. vWD type 1
B. vWD type 2A
C. vWD type 2M
D. vWD type 3
Case 1

What is the most likely diagnosis:
A. vWD type 1
B. vWD type 2A
C. vWD type 2M
D. vWD type 3
Case 1

- vWD Type 3
- Recessive disorder
- vWF protein is virtually undetectable
- Absence of vWF causes a secondary deficiency of FVIII and a subsequent severe combined defect in blood clotting and platelet adhesion
Case 2

A 6-year-old boy with frequent nosebleeds. Physical examination revealed occasional ecchymosis (subcutaneous hemorrhage / purple discoloration of the skin).
Case 2

Lab Results:

Platelet count: 360 K/ul (229-465 K/ul)
aPTT (Partial Thromboplastin Time): 30 s (24-35 s)

vWF:Ag       21% (0-6 years: 52-214%)
vWF:RCo       20% (0-6 years: 51-215%)
Factor VIII   60% (0-6 years: 56-191%)
Case 2

Ctrl  Patient
Case 2

What is the most likely diagnosis:
A. vWD type 1
B. vWD type 2A
C. vWD type 2B
D. vWD type 3
Case 2

What is the most likely diagnosis:

A. vWD type 1
B. vWD type 2A
C. vWD type 2B
D. vWD type 3
Case 2

- vWD Type 1
- Mild to moderate disease
- Mild quantitative deficiency of vWF
- vWF is functionally normal
- Usually autosomal dominant
Case 3

A 5-year-old boy recent had gingival bleeding and nosebleed.

Lab Results:

Platelet count: 289 K/ul (229-465 K/ul)
aPTT (Partial Thromboplastin Time): 85 s (24-35 s)

vWF:Ag    156% (0-6 years: 52-214%)
vWF:RCo    135% (0-6 years: 51-215%)
Factor VIII 8% (0-6 years: 56-191%)

Genetic testing ruled out Hemophilia A (FVIII deficiency)
Case 3

What is the most likely diagnosis:
A. vWD type 1
B. vWD type 2A
C. vWD type 2N
D. vWD type 3
Case 3

What is the most likely diagnosis:

A. vWD type 1
B. vWD type 2A
**C. vWD type 2N**
D. vWD type 3
Case 3

vWD Type 2N

Markedly decreased affinity of vWF for FVIII

Results in markedly reduced FVIII level.

vWF:FVIII binding (vWF:FVIIIIB) assay
Summary

von Willebrand factor (vWF)

- Large multimeric protein
- Two major functions:
  1. Tethers the platelets to exposed collagen during injuries
  2. Serves as a carrier protein for Factor VIII
Summary

von Willebrand disease (vWD)

- Most frequent inherited bleeding disorder
- Autosomal inheritance pattern
- Quantitative: Types 1 and 3
- Qualitative: Types 2A, 2B, 2M and 2N
Summary

Laboratory tests for vWD

Initial vWD testing
- vWF:Ag
- vWF:RCo
- FVIII activity

Further testing
- vWF multimer analysis
- Ristocetin-induced platelet aggregation (RIPA)
- vWF:FVIII binding (vWF:FVIIIIB) assay
- Genetic testing
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Dr. Genzen

Dr. Fang
Dr. Hopkins
Jeanne

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References


The Diagnosis, Evaluation, and Management of von Willebrand Disease. vWD Guidelines. NIH/NHLBI


James PD and Goodeve AC. *Genetics in Medicine*. Volume 13 (5), May 2011


ARUP consult