Pitfalls in the diagnosis of VWD

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VWD: diagnosis

- Diagnosis based upon
  - Personal bleeding history (mucocutaneous bleeding)
    - Ecchymoses, gingival bleeds, epistaxis, menstrual bleeding
  - Careful with epistaxis
  - Bleeding during blood sampling, wounds, ..
  - Bleeding during and after surgery
  - Familial bleeding history
  - Laboratory abnormalities fitting VWD

Bleeding score and Bleeding Questionnaire for the Diagnosis of Type 1 von Willebrand Disease

Presentation
A physician-administered questionnaire for history-taking and bleeding score assignment in patients presenting with bleeding symptoms. Minimal clinical criteria for the identification of subjects demanding further investigations for type 1 VWD can be obtained with the use of this questionnaire and the derived quantitative assessment.

See Appendix for the criteria to be used to compute the bleeding score. 

Based on:

The questionnaire has been validated evaluating hemostatic symptoms in referral centers of type 1 VWD and comparing them with those observed in age and sex-matched normal controls. A semi-quantitative bleeding score was subsequently computed from the information collected from the questionnaire. Limitations of the questionnaire are discussed in this paper.

For further information or comments please contact:
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Kennis / Ervaring / Zorg
VWD: diagnostic problems

- **Laboratory**
  - Pre-analytical
    - Sampling technique
    - Storage and transport at 4°C can lower levels
    - Centrifugation can influence multimers
    - Freezing of plasma may influence multimers
  - Testing
    - Variability
    - Availability of tests
  - Interpretation

- **Patient-related**
  - Stress, physical activity
  - Infections
  - Hormones, pregnancy
  - Age
  - Race
  - Blood group
Problems with laboratory testing

- Storing and transport of samples at 4°C can importantly lower VWF levels.

![Graph showing assay results for vWF:Ag, vWF:RCO, vWF:CB, FVIII:C at 22°C and 4°C.]

Problems with laboratory testing

- Large variation in test results
  - VWF:RCo  CV 25-30%
  - VWF:CB  CV 15-25%

Problems with laboratory testing

## VWD: molecular analysis

- **Linkage**
- **ISTH-SSC**

<table>
<thead>
<tr>
<th>Type</th>
<th>N</th>
<th>Mutation</th>
<th>Phenomenon</th>
<th>Location</th>
<th>Age</th>
<th>Classification</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>26</td>
<td>3430T&gt;G</td>
<td>W1144G</td>
<td>USA</td>
<td>Yes</td>
<td>Montgomery</td>
<td>By virtue of mutation, VWF clearance is less than 3 hours.</td>
</tr>
<tr>
<td>Type 1</td>
<td>26</td>
<td>3430T&gt;G</td>
<td>W1144G</td>
<td>EU</td>
<td>No</td>
<td>MCDMD-1VWD</td>
<td>Mutation associated with an abnormal multimer pattern. Previously classified as a type 1A mutation, detailed multimer analysis reclassified it as a type A mutation.</td>
</tr>
<tr>
<td>Type 2A</td>
<td>26</td>
<td>3430T&gt;G</td>
<td>W1144G</td>
<td>EU</td>
<td>No</td>
<td>MCDMD-1VWD</td>
<td>Mutation associated with an abnormal multimer pattern. Previously classified as a type 1A mutation, detailed multimer analysis reclassified it as a type 2A mutation.</td>
</tr>
<tr>
<td>Type 1</td>
<td>26</td>
<td>3437A&gt;G</td>
<td>Y1146C</td>
<td>Canada</td>
<td>No</td>
<td>Lillicrap</td>
<td>VWF:Ag 0.20 +/- 0.07; VWF:CB 0.16 +/- 0.06; VWF:RCo 0.07 +/- 0.03 FVIII:C 0.35 +/- 0.31 (17 individual, 12 IC). Functional studies showed</td>
</tr>
<tr>
<td>Type 2A</td>
<td>26</td>
<td>3437A&gt;G</td>
<td>Y1146C</td>
<td>Germany</td>
<td>Yes</td>
<td>Schneppenheim</td>
<td></td>
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</tbody>
</table>
VWD & influencing factors

• **Age**
  - Neonates are born with higher levels
  - Children are often stressed for blood sampling
    - Complicates diagnosis of mild type 1
  - VWF rises slowly with age (10% per decade)

• **Stress & physical activity**
  - Physical and mental stress increase VWF levels
  - Increased levels can stay increased for up to 10 hrs
  - Crying in children leads to higher VWF levels

Especially mild VWD type 1 can easily be missed!
  - Repetition of blood testing is advised if clear bleeding history
VWF and physical exercise

- Step exercise at 32 steps/minute

Figure 1: Mean absolute values of vWF:Ag (U/ml) and FVIII:C (U/ml) at baseline (Time 0), immediately after exercise (Time 1) and 30 minutes after exercise (Time 2).

Figure 2: Mean increases (in per cent) in vWF:Ag and FVIII:C immediately after exercise (Time 1), and 30 minutes after exercise (Time 2) adjusted for baseline (Time 0).

• 10 healthy young males 22-30y
  • Arithmetic and color word test (20 mins)
  • Physical exercise
  • Adrenaline infusion

• 25% difference between O and non-O
• Large overlap between normal values for O and VWD in O patients

Over-diagnosis of VWD in blood group O
Case 1: DDB °1960

- External samples (2011)
  - DDAVP test, no clinical information
- Lab results

<table>
<thead>
<tr>
<th>Test</th>
<th>Pre-DDAVP</th>
<th>Post-DDAVP</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVIII:c</td>
<td>75%</td>
<td>238%</td>
</tr>
<tr>
<td>VWF:Ag</td>
<td>79%</td>
<td>142%</td>
</tr>
<tr>
<td>VWF:RCo</td>
<td>60%</td>
<td>158%</td>
</tr>
<tr>
<td>VWF:CB</td>
<td>56%</td>
<td>190%</td>
</tr>
<tr>
<td>Bleeding Time</td>
<td>6.5 min</td>
<td>-</td>
</tr>
<tr>
<td>PFA</td>
<td>nl</td>
<td>Shortened</td>
</tr>
<tr>
<td>Whole Blood Aggregation</td>
<td>nl</td>
<td>nl</td>
</tr>
</tbody>
</table>

- Conclusion: no arguments for VWD on laboratory findings
- Molecular (exon 28):
  - p.V1229G / c.3686T>G + p.N1231T / c.3692A>C -> type 1
  - p.P1266Q / c.3797C>A -> type 2M
Case 2: JC °1986

- Patient referred to hemostasis unit
  - Easy bruising, gingival bleeding, heavy menstrual bleeding
  - Surgical removal of ovarian abces with heavy bleeding
  - Mother known with type 1 VWD
  - Referral values: FVIII 27%, VWF:Ag 38%, VWF:RCo 30%

- Laboratory
  - FVIII 101%, VWF:Ag 104%, VWF:RCo 112%
  - PFA & Multiplate aggregation tests normal
  - Multimer analysis normal
  - (Molecular analysis ongoing)

- But: Patient spent 2 hrs by train/public transport to reach hospital!
- VWD type 1 (based on referral values, personal/familial history)
Case 3: MB °1949

- External samples (2011)
- Lab results

<table>
<thead>
<tr>
<th></th>
<th>Values</th>
<th>Ratio to VWF:Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVIII:c</td>
<td>12%</td>
<td>0.80</td>
</tr>
<tr>
<td>VWF:Ag</td>
<td>15%</td>
<td>-</td>
</tr>
<tr>
<td>VWF:RCo</td>
<td>10%</td>
<td>0.67</td>
</tr>
<tr>
<td>VWF:CB</td>
<td>26%</td>
<td>1.76</td>
</tr>
<tr>
<td>VWFpp</td>
<td>73%</td>
<td>4.87</td>
</tr>
<tr>
<td>PFA</td>
<td>Epi301 / ADP301</td>
<td></td>
</tr>
<tr>
<td>RIPA 1.2</td>
<td>66%</td>
<td></td>
</tr>
<tr>
<td>VWF multimers</td>
<td>Normal, possibly ultralarge HMWM</td>
<td></td>
</tr>
<tr>
<td>Molecular analysis</td>
<td>p.R1205H / c.3614G&gt;A</td>
<td></td>
</tr>
</tbody>
</table>

- VWD type? Type 2 (VWF:Rco 0.67)? Type 1?
- Type 2M (ultralarge MM) ?
- VWD type 1 Vicenza
Case 4: MJ °1974

- Patient referred to hemostasis unit (2012)
  - Known VWD in 1 Dutch center, not found in another center
    - No VWF data, DDAVP not efficient, Haemate-P for past surgery
  - Epistaxis, bruising, tooth extraction with bleeding
  - Tonsillectomy with bleeding
  - Familial: father VWD, bleeding in paternal & maternal families
  - Blood group O
  - RFE: hernia operation 5 days later (incl weekend)
- Laboratory
- Conclusion
  - Possible type 1 VWD, but within normal limits for blood group O
  - To be repeated
  - Pragmatic: Haemate-P for planned operation (based on patient history)

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<thead>
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</thead>
<tbody>
<tr>
<td>FVIII</td>
<td>53%</td>
</tr>
<tr>
<td>VWF:Ag</td>
<td>52%</td>
</tr>
<tr>
<td>VWF:RCo</td>
<td>42%</td>
</tr>
<tr>
<td>VWF:CB</td>
<td>NA</td>
</tr>
<tr>
<td>VWF multimers</td>
<td>NA (normal)</td>
</tr>
<tr>
<td>Ivy Bleeding Time</td>
<td>7.5 min (nl)</td>
</tr>
<tr>
<td>PFA</td>
<td>normal</td>
</tr>
<tr>
<td>RIPA 1.2</td>
<td>93%</td>
</tr>
</tbody>
</table>
Pitfalls in VWD diagnosis

- Diagnosis of VWD not always easy
  - Patient circumstances
  - Laboratory issues

- Clinical picture is paramount
  - Laboratory work-up may have to be repeated

- Reliable classification may need several tests
  - Classification may vary between laboratories
    - Important issue for scientific research
    - Issue for reimbursement of medication