Challenges in the diagnosis and management of von Willebrand disease

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von Willebrand factor (VWF):

- Gene on chromosome 12p (178 kb, 52 exons): precursor molecule 2813 amino acids.
- Synthesised in endothelial cells and megakaryocytes: stored in Weibel-Palade bodies.
- VWF circulates in plasma as a series of multimers ranging from 1 - > 20 x 10^6.
- Multimers broken down by specific protease (ADAMTS 13).
- VWF required for adhesion of platelets to subendothelial tissue.
- VWF also binds factor VIII in plasma.
Rod shaped Weibel-Palade bodies (green) in endothelial cells. The periphery of the cell is shown by staining for ß-catenin (red)

Electron micrograph of Weibel-Palade bodies: longitudinal and cross-sectional views
Clinical features of VWD:

- Common but usually mild bleeding disorder
- Autosomal dominant inheritance (so females equally affected)
- Typical features include:
  - Easy bruising
  - Prolonged bleeding from cuts and scratches
  - Epistaxis
  - Menorrhagia
- Joint bleeding not a typical feature
- Diagnosis often unrecognised until middle age
- Patients often present to other specialists:
  - e.g. Gynaecologist with heavy menstrual bleeding or ENT surgeon with recurrent epistaxis
Eliciting clinical history is pivotal:

• Personal and family history
  – Bruising; bleeding after minor cuts scratches; epistaxis; menstrual periods, problems after surgery, dental extraction or childbirth; haematoma formation after i/m injections or vaccination; medication

• Bleeding score chart may be useful

• Look for objective evidence to support diagnosis of bleeding disorder:
  – Transfusion required after surgery
  – Iron supplementation or low haemoglobin level associated with heavy periods
Predictive value of symptoms in diagnosis of type 1 VWD

Simple screening questionnaire for gynaecologists to use in daily practice. Sensitivity of 89% for haemostatic defects.

**TABLE 1**

**Screening tool**

| Q1. How many days did your period usually last, from the time bleeding began until it completely stopped? |
| Q2. How often did you experience a sensation of “flooding” or “gushing” during your period? |
| Q3. During your period did you ever have bleeding where you would bleed through a tampon or napkin in ≤2 hours? |
| Q4. Have you ever been treated for anemia? |
| Q5. Has anyone in your family ever been diagnosed with a bleeding disorder? |
| Q6. Have you ever had a tooth extracted or had dental surgery? |
| Q6a. Did you have problem with bleeding after tooth extraction or dental surgery? |
| Q7. Have you ever had surgery other than dental surgery? |
| Q7a. Did you have bleeding problem after surgery? |
| Q8. Have you ever been pregnant? |
| Q8a. Have you ever had bleeding problem after delivery or after a miscarriage? |

Tests for diagnosis of VWD:

- No single test suffices for diagnosis of VWD
- Normal APTT does not exclude mild form
- Panel of tests is required:
  - Bleeding time or PFA closure time
  - Factor VIII
  - VWF antigen
  - VWF:RCo (ristocetin-induced platelet aggregation)
  - VWF:CB (collagen binding assay)
  - VWF multimer analysis
- Repeat tests if borderline
- Tendency in the past to overdiagnose VWD
Laboratory diagnosis of VWD:

• Do not be despondent if your laboratory cannot offer all diagnostic tests!

• A prolonged bleeding time and low factor VIII level are sufficient for diagnosis, when supported by suggestive clinical and family history

• VWD subtyping is not vital: what matters is identifying patients who respond to DDAVP

• Multimer analysis and genotyping are certainly not essential
Factors which influence VWF level:

- ABO group
- Secretor status
- Age
- Race
- Difficult venepuncture
- Physical exertion
- Mental stress
- Oestrogen therapy
- Menstrual cycle

- Pregnancy
- Systemic disease:
  - Malignancy
  - Infection
  - Inflammation
  - Pre-eclampsia
  - Postoperative state
  - Hypothyroidism
  - Diabetes mellitus
Effect of blood group on VWF level:

### Influence of ABO Blood Group on vWF:Ag Values in Volunteer Blood Donors

<table>
<thead>
<tr>
<th>ABO Type</th>
<th>n</th>
<th>vWF:AG Geometric Mean</th>
<th>vWF:Ag Geometric Mean ± 2 SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>456</td>
<td>74.8</td>
<td>35.6-157.0</td>
</tr>
<tr>
<td>A</td>
<td>340</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>

The groups were statistically significantly different from each other as follows: O v A, B, and AB, P < .01; A v AB, P < .01; B v A, P < .05.
UK guidelines on diagnosis of VWD:


- We recommend against the use of reference ranges or blood group-specific ranges for the diagnosis of von Willebrand disease (VWD) (2C).

- When investigating a patient with mucocutaneous bleeding a diagnosis of VWD can be made when von Willebrand factor (VWF) activity is <0.30 iu/ml (1B).

- Patients with an appropriate bleeding history and VWF activity 0.3–0.5 iu/ml should be regarded as having primary haemostatic bleeding with reduced VWF as a risk factor rather than VWD. We suggest referring to this as ‘Low VWF’ (2C).

- We recommend use of a bleeding score (e.g. Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis bleeding assessment tool) to standardize history taking (2C).

- When reviewing patients and families with an historical diagnosis of VWD, we suggest confirming the accuracy of that diagnosis (2A).

- The incidental finding of VWF activity <0.30 iu/ml should be taken to indicate VWD or acquired von Willebrand syndrome (AVWS).
Table I. Classification of VWD.

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
<th>Comments</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Partial quantitative deficiency of VWF</td>
<td>Includes VWF mutations causing rapid VWF clearance (e.g., VWF Vicenza) and requires function:antigen ratio &gt; 0.6</td>
<td>Mostly autosomal dominant inheritance when VWF &lt; 0.3 IU/ml. Mutations of VWF in kindred with levels &gt; 0.3 IU/ml show variable penetrance</td>
</tr>
<tr>
<td>2</td>
<td>Qualitative VWF defects</td>
<td>Some controversy exists regarding classification of VWF mutations associated with subtle reductions in HMW multimers</td>
<td>Mostly autosomal dominant</td>
</tr>
<tr>
<td>2A</td>
<td>Decreased VWF-dependent platelet adhesion with selective deficiency of high-molecular-weight multimers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2B</td>
<td>Increased affinity for platelet GPIb</td>
<td>Should be distinguished from PT-VWD, using either platelet agglutination tests or genetic testing. Cases with normal VWF multimer and platelet count have been described</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>2M</td>
<td>Decreased VWF-dependent platelet adhesion without selective deficiency of HMW multimers</td>
<td>This also includes defects of VWF collagen binding. May be combined quantitative/ qualitative defect</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>2N</td>
<td>Markedly decreased binding affinity for FVIII</td>
<td>Should be distinguished from mild haemophilia A</td>
<td>Reduced VWF:FVIII binding defects are more commonly identified in a compound heterozygote state with a VWF null allele rather than the classical homozygous form</td>
</tr>
<tr>
<td>3</td>
<td>Virtually complete deficiency of VWF</td>
<td>Equivalent to &lt;0.03 IU/ml in most assays</td>
<td>Autosomal recessive, frequent null VWF alleles. Bleeding symptoms in 26–48% of obligate carriers</td>
</tr>
</tbody>
</table>

VWD, von Willebrand disease; PT-VWD, platelet type pseudo-VWD; VWF, von Willebrand factor; VWF, VWF gene; FVII, factor VIII GPIb, glycoprotein Ib; HMW, high molecular weight.
UK guidelines on diagnosis of VWD:

Recommendations

• In the initial investigation for VWD, FVIII, VWF:Ag and VWF activity should be measured (1A).
• VWF activity should be assessed by its ability to bind both GPIb and collagen (2B).
• We recommend against using assays based on monoclonal antibodies directed against the VWF GPIb-binding site (1B).
• A function:antigen ratio of <0·6 should be used to identify patients with type 2 VWD (1B).
• RIPA should be performed on all patients with reduced VWF:RCo/VWF:Ag or VWF:CB/VWF:Ag ratios or when thrombocytopenia is present (1B).
• Multimer analysis should be used to distinguish between types 2A and 2M (1B).
• If multimer analysis is not available then the ratios of VWF:RCo and VWF:CB to VWF:Ag should be used to distinguish types 2A and 2M (1B).
Multimer analysis in VWD:
If only low FVIII:C, differential diagnosis is type 2N or mild haemophilia A

VWF:Ag

VWF:RCo

VWF:C

FVIII:C

Undetectable VWF:Ag

VWF:RCo and/or

VWF:CB reduced

RCo/Ag and CB/Ag

> 0.6

Type 1

RCo/Ag or CB/Ag

< 0.6

Type 2

Type 3

Response to low concentration ristocetin

Normal/reduced RIPA

Type 2B, or platelet-type

Loss of HMW multimers, RCo/Ag and CB/Ag both low

Preservation of HMW multimers, RCo/Ag low but CB/Ag normal (rarely RCo/Ag normal, CB/Ag low)

Type 2A

Type 2M

Fig 2. An algorithm for the investigation of suspected von Willebrand disease. VWF, von Willebrand factor; VWF:Ag, VWF antigen; VWF:RCo, ristocetin cofactor activity; VWF:CB, collagen binding activity; FVIII, factor VIII; FVIII:C, Factor VIII coagulant activity; GPIb, glycoprotein Ib; HMW, high molecular weight; RIPA, ristocetin-induced platelet agglutination.
Molecular basis of type 2 VWD:

www.vwf.group.shef.ac.uk/index.html

D1 D2 D’ D3A1 A2 A3 D4

chrom. 12p 178 kb 52 exons

pro-VWF

type 2N mutations

B C1 C2

aa497

909

A1 | A2

exon 28

type 2B mutations
type 2A mutations
General measures:

- Actively seek out other possible affected individuals in the extended family
  - Draw up family tree
  - Screening of very young children is best deferred
- Register on national database (if applicable)
- Issue bleeding disorders card
- Tell patient to avoid aspirin and NSAIDs in future+
- Tranexamic acid useful for mucosal bleeds
- Avoid intramuscular injections in future
  - Vaccinations to be given by s/c injection
- Emphasise importance of travel insurance for trips abroad
Desmopressin (DDAVP):

- Analogue of natural antidiuretic hormone (ADH)
- Boosts levels of factor VIII and VWF (no effect on IX level)
- Cheap and free of risk of viral transmission
- 0.3 µg/kg is usual dose by s/c injection: peak effect seen after one hour
- Very useful in mild haemophilia A, female carriers and most cases of von Willebrand disease
- Generally well tolerated: hyponatraemia and arterial thrombosis are rare complications
  - Avoid use in elderly and children under age of 2
  - Limit fluid intake to 1 litre for 24 hours after dose
- Effect wears off with repeated dosing over several successive days (tachyphylaxis)
Response to desmopressin in different types of VWD:

<table>
<thead>
<tr>
<th>Type</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Usually effective</td>
</tr>
<tr>
<td>2A</td>
<td>Usually ineffective</td>
</tr>
<tr>
<td>2B</td>
<td>May be contraindicated</td>
</tr>
<tr>
<td>2M</td>
<td>Predicted to be ineffective</td>
</tr>
<tr>
<td>2N</td>
<td>Rarely effective</td>
</tr>
<tr>
<td>3</td>
<td>Ineffective</td>
</tr>
</tbody>
</table>

Various formulations of DDAVP exist: make sure you use the correct one!

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>Suggested formulation</th>
<th>Dose</th>
<th>Peak levels achieved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous infusion</td>
<td>4 µg/ml diluted in 100 ml 0.9% NaCl for</td>
<td>0.3 µg/kg</td>
<td>15 min after infusion completed</td>
</tr>
<tr>
<td>30–60 min</td>
<td>infusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>15 µg/ml</td>
<td>0.3 µg/kg</td>
<td>60–90 min after injection</td>
</tr>
<tr>
<td>Intranasal</td>
<td>150 µg per metered spray</td>
<td>&gt;50 kg: 150 µg spray to each nostril</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>≤50 kg: single 150 µg spray</td>
<td></td>
</tr>
</tbody>
</table>
Response to DDAVP in VWD:
Mannucci PM: Br. J. Haematol. 82: 87-93 (1992)

Fig 3. Ristocetin cofactor activity. See also Fig 2.

Fig 4. Bleeding time. See also Fig 2.
Surgery in VWD:

• Minor surgery in patients with type 1 VWD may be feasible with DDAVP alone
• Major procedures that require prolonged correction of factor VIII level will require treatment with blood products
• FVIII level predictive risk of bleeding
• Correction of bleeding time is not always observed and is not essential
• Cryoprecipitate may be used but is far from ideal
• High-purity plasma or recombinant FVIII products alone of no value
Surgery in VWD:

- Raise factor VIII level to ≈ 1 iu/ml peri-operatively, and maintain at or above 0.50 iu/ml until wound healing complete
- Commonly used concentrates include: Wilate (Octapharma); Voncento (CSL Behring), Alphanate (Grifols); Wilfactin (LFB); 8Y (BPL)
- Recombinant VWF concentrate now available (Vonvendi, Shire)
- Venous thromboembolism has been reported in some patients with VWD undergoing surgery
  - Associated with high levels of factor VIII
  - Use some form of thromboprophylaxis (physical and/or chemical)
Pregnancy in women with VWD:

- Haemostatic support seldom needed as VWF levels generally rise to within normal range by term
- Check factor levels at 34-36 weeks
- Vaginal delivery generally regarded as safe if VWF activity is $\geq 0.50$ iu/ml
- Similar threshold for Caesarean section and epidural
- Avoid aspirin-like analgesics
- Cord blood screening of baby is unlikely to yield reliable results in type 1 VWD
- Increased risk of post-partum haemorrhage ($\approx 20\%$)
  - VWF starts to fall towards baseline at day 3 (before FVIII)

DDAVP in pregnancy:

- $V_2$ agonist: little pressor or oxytocic effect
- Manufacturers still advise “use with caution” in pregnancy
- No problem after delivery, with cord clamped
- Peptide does not pass into breast milk
- Growing experience of safe use in pregnancy:
  - 32 cases, mainly to cover CVS or fetal blood sampling (11-18 weeks gestation)
  - 54 cases: used throughout pregnancy
  - Dose used in both series was standard 0.3 µg/kg
Type 2N VWD:

- Often mistaken for mild haemophilia A
- Factor VIII typically in range 3-15 iu/dl (%)
- VWF level normal in all assays (Ag/RCo/CB)
- Bleeding time is normal
- Defect is in N-terminal of VWF, so factor VIII binding to VWF is impaired
  - Three mutations in exons 18-20 account for ≈ 95% of cases: T791M, R816W, R854Q
- Autosomal recessive inheritance: both sexes affected in family tree
Type 2N VWD:

• DNA-based genetic studies will confirm diagnosis
  – Factor VIII binding assay is alternative test
• DDAVP and high-purity or recombinant factor VIII products only provide a limited and transient boost of factor VIII levels and should not be used
• Best treatment is a plasma-derived factor VIII concentrate which contains VWF as well (such as Voncento, Wilate, 8Y, Alphanate)
• Always consider alternative possibility of type 2N VWD in apparent cases of mild haemophilia A
A baby with severe type 3 von Willebrand disease:
Type 3 VWD:

- Virtual or complete absence of VWF
- Rare: just 1-2% of all patients of VWD
- Most patients have surprisingly mild bleeding manifestations compared to haemophilia
  - Two bleeds/year typically observed in Italian cohort of 56 patients with type 3 VWD *Federici A et al. Blood 110: 713 (2007)*
  - Soft tissue and joints bleeding can occur in this type of VWD due to very low FVIII levels
  - Other common problems include epistaxis, heavy menstrual blood loss, oral bleeding and gastrointestinal bleeding
- Prophylaxis may be useful: 20-40 iu/kg 1-3 times weekly
Type 3 VWD:

- Alloantibodies are an occasional complication
  - Incidence 8% in one survey of 150 patients (Mannucci, 1995)
  - Typically arise after multiple transfusions
  - Serious allergic reactions frequently reported
- Often difficult to detect inhibitory antibodies
  - Inhibitory antibodies often fully saturated in complexes with VWF so no free antibodies left
  - Probable that low titre inhibitors are underdiagnosed
- Plasma concentrates containing VWF should not be given if presence of inhibitors is suspected
- Recommended treatment is recombinant activated factor VII: same dose regime as used for haemophilia
Fig 1. Angiodysplasia of the ileum as seen by video-endoscopy in a 70-year-old patient with type 3 von Willebrand disease and recurrent gastrointestinal bleeding.
Gastrointestinal angiodysplasia:  

- Can develop in with types 2A and 3 VWD  
  - Incidence about 3% in one large survey (Fressinaud, 1993)
- Seems to be a direct result of lack of VWF HMW in Weibel Palade bodies, resulting in abnormal angiogenesis throughout GI tract and recurrent bleeding
- Challenging clinical problem:  
  - Surgery will not cure the problem  
  - Prophylaxis with VWF concentrate may help  
  - Recurrent transfusion results in alloimmunization  
  - Tranexamic acid also worthwhile
- Case reports of improvement on octreotide, atorvastatin and lenalidomide

Fig 2. (Courtesy of Anna Maria Randi and Richard Starke): A model explaining how von Willebrand factor (VWF) may control the angiopoietin-Tie-2 pathway and angiogenesis. In stable, mature blood vessels, angiopoietin-1 (Ang-1) is produced by mural cell signals through the Tie-2 receptors and promotes stability of the endothelial cell (EC) monolayer and its quiescence. Ang-2 can antagonize Ang-1 signalling and promote destabilization of junctions and angiogenesis. Ang-2 is normally stored in Weibel Palade Bodies (WPB), together with VWF and several other components. Because VWF is required for WPB assembly, a decrease in VWF levels in EC results in released depletion of Ang-2 from the EC, which can then bind to the Tie-2 receptor and antagonise the effects of Ang-1.
Acquired VWD

- Often confused with congenital VWD
- Most frequently diagnosed in the elderly: median age 62 (range 2-96) in ISTH registry
- Underlying disorder usually present:
  - Lymphoproliferative 48%
  - Myeloproliferative 15%
  - Other neoplastic 5%
  - Autoimmune 2%
  - Cardiovascular 21% (data from ISTH registry)
Acquired VWD:

• In majority of cases, specific antibodies or non-specific proteins result in increased clearance of VWF
• Treatment of underlying condition is key to resolution
• A minority of cases not associated with inhibitory antibodies:
  – Hypothyroidism
  – Sodium valproate therapy
  – Cardiovascular diseases (esp. aortic stenosis; congenital shunts and stenoses; cardiac assist devices)
• Initial laboratory findings generally indistinguishable from congenital VWD
• Previous personal history and family history may help to distinguish
Diagnosis of acquired VWD:

- Failure to consider possibility of this disorder is the major obstacle to identification
- Initial tests are exactly the same as those used to identify congenital VWD
- Very difficult to identify inhibitory antibodies with conventional mixing studies
  - Often saturated in complexes with VWF so no free antibodies remaining in plasma
- Multimer analysis often shows unusual patterns and triplet structure
- Platelet function may be disproportionately abnormal
Principal conclusions:

• VWD is commonest inherited bleeding disorder
• No single test exists for diagnosis
  – Be aware of factors which can modify VWF levels
  – Repeat testing may be need in borderline cases
  – Use of PFA preferable to bleeding time
  – Multimer analysis is not essential
• Use threshold of 0.3 iu/ml VWF for diagnosis of VWD
• Invest time in educating colleagues in other specialities who may see these patients first
• Trial of DDAVP is warranted in types 1 & 2A/M
• Always consider alternative diagnosis of 2N VWD in apparent cases of mild haemophilia
• The possibility of acquired VWD should always be considered, especially in older patients
Advances in the diagnosis and treatment of Von Willebrand disease

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¹Pediatric Hematology/Oncology, Medical College of Wisconsin, Milwaukee, WI; ²Blood Research Institute, BloodCenter of Wisconsin, Milwaukee, WI; and ³Children’s Research Institute, Children’s Hospital of Wisconsin, Milwaukee, WI

Von Willebrand disease (VWD) is the most common inherited bleeding disorder, yet diagnosis and management remain challenging. Development and use of bleeding assessment tools allows for improved stratification of which patients may require further assessment and which patients are most likely to require treatment of their VWD. New options for laboratory assessment of von Willebrand factor (VWF) activity include a new platelet-binding assay, the VWF:GPIbM, which is subject to less variability than the ristocetin cofactor activity assay, and collagen-binding assays that provide insight into a different function of VWF. Genetic testing may be helpful in some cases where a type 2 VWD variant is suspected but is usually not helpful in type 1 VWD. Finally, treatment options for VWD are reviewed, including the use of recombinant VWF. Despite these advances, still more work is required to improve diagnosis, treatment, and quality of life for affected patients. (Blood. 2017;130(22):2386-2391)

Blood 130: 2386-2391 (2017)