Accurate diagnosis of high-affinity vWF-platelet disorders: a case study of pseudo von Willebrand disease

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Abstract
A pregnant woman with a family history of abnormal bleeding was referred to our laboratory for investigation. Abnormal PFA-100 results were initially found and von Willebrand Factor (vWF) studies indicated a functional discordance (ie functional assays of vWF being significantly reduced as compared to vWF:Ag) suggestive of type 2 von Willebrand Disease (vWD). Further testing raised doubts that this was a ‘typical’ case of type 2B vWD and may in fact be pseudo (platelet type) vWD. Pseudo vWD was confirmed after in-depth laboratory testing and this case study demonstrates that this very rare sub-type of vWD may be under diagnosed due to its phenotypic similarities to type 2B vWD.

Key words: pseudo von Willebrand disease; platelet aggregation; PFA-100; RIPA; platelet glycoprotein-1b receptor.


Case history
A 24 year old woman presented at 36 weeks of pregnancy due to a maternal history of bleeding. The patient's mother had suffered a post partum haemorrhage controlled with utero-tonic medication and also a maternal cousin resident in Australia had been diagnosed with vWD (no data available). The patient had a normal platelet count which is not usually the case in type 2B vWD. vWF multimer analysis (Figure 2) showed that high, intermediate and low molecular weight multimers were present but a slight reduction in the assays which measure vWF function (vWF:RCo and vWF:CB) indicating a possible functional vWF defect was the cause of the high affinity platelet–vWF interaction. Type 2B vWD was a likely diagnosis although this pattern of results was also not consistent with type 2B vWD. With vWF levels intermediate and low molecular weight multimers were present, high, intermediate and low concentration Ristocetin is shown in Figure 1b). The patient went on to receive DDAVP prior to epidural catheter insertion and some hours later underwent caesarean section for failure to progress. There was some excess incision site bleeding noticed in the post-operative period and Biostate was administered with good results. The patient then demonstrated an uneventful recovery and the child was well.

Six weeks post partum the patient returned for further investigations. PFA-100, vWF studies, vWF multimers and Ristocetin Induced Platelet Aggregation (RIPA) were repeated with results confirming the previous findings (Table 1) except that vWF parameters were now more significantly reduced but still demonstrated the functional discordance.

Type 2B vWD was a likely diagnosis although this pattern of results was found only in pseudo vWD. To differentiate these two disorders RIPA mixing studies were performed. RIPA mixing studies involve performing RIPA on a combination of:

(i) patient platelets + patient plasma
(ii) patient platelets + control plasma
(iii) control platelets + control plasma
(iv) control platelets + patient plasma

Enhanced response to low concentration Ristocetin was found only in the presence of patient platelets indicating an intrinsic platelet defect was the cause of the high affinity platelet–vWF interaction. Therefore the diagnosis was pseudo (platelet type) vWD which is a rare disorder arising from a gain of function mutation in the gene for the platelet glycoprotein-1b receptor (GP1BA).

To confirm this diagnosis mutation analysis of the GP1BA gene was performed. Coding regions and flanking intronic sequences of the GP1BA gene were amplified by PCR and analysed by bi-directional automated sequencing. The sequence was then compared to the reference genomic DNA sequence for GP1BA (GenBank NT010718). A mutation c.746G>T was identified which results in the substitution p.G249S (G233S in old nomenclature) and is known to cause pseudo vWD (2).

Subsequent genetic analysis was performed on the patient's child and the mutation found in the mother was also found in the child. Due to the large amount of blood required phenotypic analysis has not been performed yet.

Some details of this case can also be found in another publication on a review of pseudo vWD and its diagnosis (3).

Discussion
Platelet–vWF interaction in the process of platelet adhesion involves optimal binding of the A1 domain of vWF protein with the GP Ib in the platelet membrane GPIb-IX-V receptor complex. Abnormally increased affinity between this receptor and ligand results in spontaneous binding of high molecular weight multimers (HMWM) of vWF to platelets. Increased HMWM vWF clearance, spontaneous platelet agglutination and variable thrombocytopenia ensue. When considering a high affinity vWD with discordant parameters for vWF:Ag and function, the diagnostic strategy should follow a logical stepwise sequence. The RIPA procedure using patient platelet rich plasma should be performed using standard (1.5mg/ml) and low (0.5mg/ml) concentration Ristocetin. Aggregation to 0.5mg/ml Ristocetin is abnormal confirming a high affinity interaction. The next step involves mixing studies, designed to separately explore the two components of the high affinity interaction: the patient’s platelets and vWF plasma protein, as a mutation in either can result in this phenotype.
Pseudo vWD is a rare disorder arising from a gain of function mutation in the gene for GP1BA as first described in 1982 (3). Type 2B first described in 1980 (4) is caused by functionally defective vWF with high affinity for GP1BA resulting from a mutation in the vWF gene located on chromosome 12. Patients with these bleeding disorders have similar phenotypic parameters and clinical features, ie functional VWF discordance, increased response to low concentration Ristocetin in RIPA testing and in most cases although not ours thrombocytopenia and loss of HMWM. Incidence of pseudo vWD is unknown but it is feasible that under diagnosis may be evident (5) as some patients previously classified as type 2B vWD may in fact have pseudo vWD.

Correct distinction between these two disorders has important clinical implications. Treatment of type 2B vWD is usually with a virucidally–treated plasma derived vWF/FVIII concentrate; treatment of pseudo vWD may require platelet transfusion.

**Conclusion**
This case study highlights the need that if a patient presents with a functional vWF discordance and enhanced response to low concentration Ristocetin in RIPA testing, a diagnosis of type 2B vWD should not be automatically assumed. Further testing by RIPA mixing studies and appropriately targeted gene mutation study is necessary for correct diagnosis.

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<tr>
<th>Table 1. Laboratory results</th>
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<tr>
<td><strong>Assay</strong></td>
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<tr>
<td>PFA-100 (COL/EPI)</td>
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<tr>
<td>PFA-100(COL/ADP)</td>
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<td>FVIII:C</td>
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<td>vWF:Ag</td>
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<td>vWF:RCo</td>
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<td>vWF:CB</td>
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<td>Platelet count</td>
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<td>VWF Multimers</td>
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**Figure 1a.** Patient RIPA results – shows abnormal response to 0.5mg/mL Ristocetin

| **Figure 1b.** Control RIPA results - shows normal response to 0.5mg/mL Ristocetin |
| Green tracing – Ristocetin 1.5mg/mL. Red tracing – Ristocetin 1.0mg/mL. Blue tracing – Ristocetin 0.5mg/mL |
Figure 2. VWF Multimer Analysis

References

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