Platelet von Willebrand Factor: An Important Determinant of the Bleeding Time in Type I von Willebrand’s Disease

By Harvey R. Gralnick, Margaret E. Rick, Laurie P. McKeown, Sybil B. Williams, Robert I. Parker, Pascal Maisonneuve, Christine Jenneau, and Yvette Sultan

We studied 17 patients with moderate to mild type I von Willebrand’s disease (vWd) and correlated the bleeding time with the plasma von Willebrand factor antigen (vWf Ag), the plasma vWf activity (ristocetin cofactor), the platelet vWf Ag, and the platelet vWf activity. We found an excellent correlation between the bleeding time and the platelet vWf activity and, to a lesser extent, between the bleeding time and the platelet vWf Ag. The length of the bleeding time was inversely proportional to the level of the platelet vWf (P < .001) or, to a lesser extent, the platelet vWf Ag (P < .05). The plasma vWf Ag and activity did not correlate significantly with the bleeding time. These studies indicate that the platelet vWf is one of the important bleeding time factors in type I vWd and that the platelet vWf plays an important role in the early steps of hemostasis.

The congenital coagulation disorder, von Willebrand’s disease (vWd), is characterized by a long bleeding time, low levels of factor VIII coagulant activity (VIII:C), von Willebrand factor antigen (vWf Ag), and ristocetin cofactor activity (vWf activity). The most common form of vWd, ie, type I, is inherited in an autosomal manner and has a concordant reduction of all of the above mentioned activities. However, the bleeding time is quite variable and often does not correlate well with the VIII:C, plasma vWf Ag and/or, in general, with the ristocetin cofactor activity (RCof). This has led some investigators to suggest that a separate bleeding time factor exists that is deficient in vWd. In an attempt to identify a factor that might be related to the bleeding time prolongation in vWd, we studied 21 patients with type I vWd (including four patients with severe disease and 17 patients with moderate to mild clinical symptoms) and correlated the bleeding time with the plasma and platelet vWf Ag and activity as measured by the RCof assay. In this study, we found that a prolonged bleeding time had an excellent correlation with a decreased level of platelet vWf activity and that a normal bleeding time correlated with normal platelet vWf activity. The plasma vWf Ag and vWf activity showed a poor correlation with the bleeding time values.

These studies indicate that an important determinant of bleeding time in type I vWd is the platelet vWf activity and suggest that it plays an important role in the initial phases of the formation of the hemostatic plug.

Materials and Methods

Each patient gave informed consent for these studies. None of the patients were taking drugs known to affect factor VIII or vWf Ag or activity. Blood was obtained with a 19-gauge needle using a two-syringe technique. Plasma preparation and separation of platelets from whole blood were performed as previously described. Platelets were isolated from whole blood anticoagulated with 10.9 mmol/L of sodium citrate, 1 mmol/L of EDTA by centrifugation on a discontinuous arabinogalactan (Stractan, St. Regis Co., Takoma, Wash) gradient (3 mL 20% of 5 mL 10% Stractan) as previously described. In some experiments, 5 mmol/L of EDTA, 6 mmol/L N-ethylmaleimide, and 1 mmol/L of leupeptin were added to the anticoagulant used for the separation of plasma and platelets. Platelets were washed free of Stractan with 0.01 mol/L of Tris, 0.15 mol/L of NaCl, 0.003 mol/L of EDTA, and 0.5% serum bovine albumin (BSA), pH 7.35, incubated at 37°C for 15 minutes, and then counted. The platelet count was adjusted to 10^6 platelets per microliter by the addition of resuspending buffer, and the platelets were lysed by the addition of 1/40 vol of 20% wt/wt Triton X-100. The platelets were then frozen at -70°C, and analysis was then thawed and spun at 10,000 g for 30 minutes at 4°C. The supernatant was removed and used in the assays for vWf Ag and vWf activity.

Platelet VIII:C activity, plasma and platelet vWf Ag, and vWf activity were measured as previously described. One unit of activity is defined as that found in 1 mL of pooled normal plasma. The bleeding time was performed on the patients’ forearms using a standardized template method. Bleeding times and plasma and platelet vWf studies were performed on at least two separate occasions.

The multimeric structure of the plasma and platelet vWf were studied by glyoxal agarose gel electrophoresis modified from the technique originally described by Hoyer and Shainoff. The major modifications were a gel thickness of 0.5 mm and a final sample size of 10 μL. Equal volumes of normal and patient plasma and platelet lysate were electrophoresed on the gel (regardless of the antigen content). Three of the four patients with severe type I vWd had no family history of a bleeding disorder, whereas one patient had one family member (brother) who died of hemorrhage without a diagnosis ever being established. The other 17 patients with type I vWd had a personal history of bleeding and had at least one immediate family member with a similar bleeding history.

Classical correlation coefficients were determined on a Model 10 Hewlett-Packard programmable computer.

Results

The four patients with severe homozygous or double heterozygous vWd were shown to have plasma and platelet vWf Ag and vWf activity of <0.03 U/mL (Figs 1 and 2) and bleeding times >30 minutes. In contrast, the 17 patients with moderate to mild type I vWd had bleeding times that ranged from 4 to 27 minutes and VIII:C levels that varied between 0.08 to 0.75 U/mL. The plasma vWf Ag ranged between 0.08 to 0.75 U/mL.
The correlation coefficients determined for the 17 patients with moderate to mild vWD are as follows. There was no correlation between the bleeding time and the VIII:C (data not shown), nor was there a correlation between the bleeding time and the plasma vWF Ag ($r = .2034$, $P > .10$) or vWF activity ($r = .1677$, $P > .10$) (Fig 1 and Table 1). In contrast, the relationship between the platelet vWF activity and the bleeding time revealed a remarkably good correlation ($r = .8055$, $P < .001$), whereas the correlation of the bleeding time with the platelet vWF Ag was significant but less than that observed with the platelet vWF activity ($r = .4895$, $P < .05$). The results of the duplicate analyses were within 1 to 2 minutes for the bleeding time and 10% to 16% for the plasma and platelet vWF studies.

The five patients who had decreased amounts of platelet vWF activity all had prolonged bleeding times, whereas 10 of the 12 patients with normal platelet vWF activity had normal bleeding times (Fig 2). The two patients who had normal levels of platelet vWF activity and prolonged bleeding times were somewhat unique. One of these patients (patient 4, Table 1) had a bleeding time of 17 minutes despite having a platelet vWF of $0.70 U/10^8$ platelets. When he was given 1-deamino-8-D-arginine vasopressin (DDAVP), his bleeding time shortened to 10 minutes with a rise in his plasma VIII:C, vWF Ag, and RCof activity to normal levels. Later, he was restudied and was shown to have a platelet storage pool defect. The second patient had normal levels of vWF Ag and vWF activity in her platelets but had the most severe deficiency of plasma vWF (0.05 U/mL) and factor VIII:C. This woman had a bleeding time of 12.5 minutes, much shorter than would be expected from her plasma vWF levels. She had undergone five major surgical procedures (including a tonsillectomy, hysterectomy, and two spontaneous deliveries) with no excessive bleeding. Of the 17 patients with type I vWD listed in Table 1, patients 14, 15, and 16 are the father and two sons in one family, respectively; and patients 6 and 11 are the mother and son from another family.

Glyoxal agarose electrophoresis of the plasma and platelet vWF in the 17 type I patients revealed a multimeric pattern qualitatively indistinguishable from normal. There appeared to be a marked decrease in the plasma vWF Ag content in almost all patients and in the platelet vWF content of five patients (Fig 3). The presence of protease inhibitors in the anticoagulant did not modify the multimeric organization, vWF Ag levels, or vWF activity of the plasma or platelets.

**DISCUSSION**

In the first description of vWD, Willebrand noted that all of the affected family members had prolonged bleeding times; this became a prerequisite for the diagnosis of the disease which bears his name.1 A 1968 study of 37 patients with vWD revealed that two patients had normal bleeding times, and a large study in 1977 revealed that 15 of 83 vWD patients with disease of moderate to clinical severity had normal bleeding times.4,10 One study has suggested a good correlation between plasma vWF activity and the bleeding time,11 whereas other investigators have found a poor correlation between the bleeding time and the plasma vWF Ag or
Table 1. Type I von Willebrand’s Disease, Dominant Inheritance

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Bleeding Time (min)</th>
<th>F.VIII (U/mL)</th>
<th>vWF Antigen (U/mL)</th>
<th>vWF Activity (U/mL)</th>
<th>vWF Antigen (U/10^6)</th>
<th>vWF Activity (U/10^6)</th>
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<tr>
<td>1</td>
<td>27</td>
<td>0.09</td>
<td>0.12</td>
<td>0.10</td>
<td>0.22</td>
<td>0.10</td>
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<tr>
<td>2</td>
<td>20</td>
<td>0.40</td>
<td>0.23</td>
<td>0.27</td>
<td>0.25</td>
<td>0.20</td>
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<tr>
<td>3</td>
<td>18</td>
<td>0.47</td>
<td>0.70</td>
<td>0.70</td>
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<td>0.68</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>0.54</td>
<td>0.32</td>
<td>0.38</td>
<td>0.46</td>
<td>0.23</td>
</tr>
<tr>
<td>5</td>
<td>16</td>
<td>0.30</td>
<td>0.32</td>
<td>0.23</td>
<td>0.35</td>
<td>0.30</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>0.31</td>
<td>0.46</td>
<td>0.44</td>
<td>0.37</td>
<td>0.63</td>
</tr>
<tr>
<td>7</td>
<td>12.5</td>
<td>0.08</td>
<td>0.11</td>
<td>0.05</td>
<td>0.36</td>
<td>0.70</td>
</tr>
<tr>
<td>8</td>
<td>7.5</td>
<td>0.59</td>
<td>0.45</td>
<td>0.28</td>
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<td>0.82</td>
</tr>
<tr>
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<td>7</td>
<td>0.47</td>
<td>0.27</td>
<td>0.29</td>
<td>1.30</td>
<td>0.98</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>0.29</td>
<td>0.48</td>
<td>0.68</td>
<td>0.25</td>
<td>0.50</td>
</tr>
<tr>
<td>11</td>
<td>6</td>
<td>0.16</td>
<td>0.14</td>
<td>0.13</td>
<td>0.60</td>
<td>1.25</td>
</tr>
<tr>
<td>12</td>
<td>6</td>
<td>0.41</td>
<td>0.46</td>
<td>0.21</td>
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<td>0.47</td>
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<tr>
<td>13</td>
<td>6</td>
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<td>5</td>
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<td>0.12</td>
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<td>1.07</td>
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<tr>
<td>15</td>
<td>4</td>
<td>0.17</td>
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<td>0.10</td>
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<td>1.65</td>
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<tr>
<td>16</td>
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<td>0.46</td>
<td>1.75</td>
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<tr>
<td>17</td>
<td>4</td>
<td>0.75</td>
<td>0.28</td>
<td>0.40</td>
<td>0.52-1.58</td>
<td>0.55-2.13</td>
</tr>
</tbody>
</table>

Normal range: F.VIII <8 U/mL; vWF Antigen <0.5 U/10^6; vWF Activity <0.5 U/10^6.

*Units per 10^6 platelets.
†Studies on 20 normal subjects: 10 male, 10 female.

The inability to find a constant correlation between laboratory parameters and the bleeding time led to speculation concerning the factor(s) involved in the bleeding time in vWD.1,2 The inability to find a constant correlation between laboratory parameters and the bleeding time led to speculation concerning the factor(s) involved in the bleeding time in vWD.1,2 The inability to find a constant correlation between laboratory parameters and the bleeding time led to speculation concerning the factor(s) involved in the bleeding time in vWD.1,2

We studied 17 patients with heterozygous type I vWD and found an excellent correlation between the level of the platelet vWF activity and the bleeding time; the correlation between the platelet vWF Ag and the bleeding time was significant but not as strong as that seen with the platelet vWF activity. In contrast, the plasma levels of vWF Ag and/or activity did not correlate with the bleeding time. These studies indicate that the platelet vWF is a major factor in determining the bleeding time in the nontreated state in type I vWD. Our studies do not exclude an additive or independent role for plasma vWF but indicate that it appears to play a less significant role than that of the platelet vWF.

Other studies reported subsets of patients with type I vWD who had normal platelet vWF Ag and/or activity,12 but no correlation was made between these values and any clinical parameter until recently. Mannucci and co-workers recently described six patients with type I vWD who had normal platelet vWF Ag and vWF activity. Two of these patients had normal resting bleeding times, and all six patients had a normal bleeding time after DDAVP infusions. The patients with type I platelet low or platelet discordant vWD had prolonged bleeding times, and although the bleeding time shortened after DDAVP infusion, it did not become normal. Ratnoff and Bennett reported that the bleeding time remained prolonged in three vWD patients in whom the VIII:C, vWF Ag, and plasma vWF activity rose to normal. It seems likely that in those patients the platelet vWF was still diminished and not affected by the pregnant state, by plasma product transfusion, or by the stress of hemorrhage.

Our studies suggest that the platelet vWF is an important determinant in the bleeding time and that either the platelet membrane vWF or the vWF which is released after platelet stimulation acts as the initial bridge between platelets and the vessel wall, causing adhesion of these platelets to the vessel, or facilitates platelet–platelet interaction after adherence to the vessel wall.12
The findings of decreased levels of plasma vWF Ag and activity and normal or increased platelet vWF Ag and activity in some type I patients suggests that the genetic defect(s) that causes a decrease in the plasma vWF protein and its activities does not necessarily affect the platelet vWF and its activities. Further studies of type I vWD patients should include measurement of platelet vWF Ag and vWF activity. These studies focus on the importance of the platelet vWF activity in the determination of the bleeding time in vWD during a steady state. Further studies are necessary to identify the source and quantity of the vWF bound to subendothelium and the importance of the endothelial cell vWF and other cytoadhesive proteins in the determination of the bleeding time.

REFERENCES

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HR Gralnick, ME Rick, LP McKeown, SB Williams, RI Parker, P Maisonneuve, C Jenneau and Y Sultan