A Syndrome of Platelet-release Abnormality and Mild Hemophilia

By Carolyn Chesney, Robert W. Colman, and Liberto Pechet

Two families were studied because of a hemorrhagic tendency. The presumptive diagnosis of von Willebrand's disease was suggested by low factor VIII levels (7.5%–33%), prolonged template Ivy bleeding time (9.5–17 min), low platelet adhesiveness (0%–8%), normal platelet factor 3, and normal clot retraction. Further studies, however, showed abnormal platelet aggregation with ADP, epinephrine, and collagen, and deficient release of platelet antihematin activity and 14C serotonin. Patients' platelets, rendered free of plasma by gel filtration, continued to show abnormal aggregation when resuspended in normal plasma. Plasma from the patients contained greater than 200% factor VIII by immunologic assay. The patients' coagulant factor VIII level returned to baseline within 24 hr after plasma or cryoprecipitate transfusions. Unlike von Willebrand's disease, management of these patients required both platelets and cryoprecipitate to prevent bleeding.

Despite the genetic, clinical, and immunoechemical differences between von Willebrand's disease and classic hemophilia, puzzling cases have been reported in which the distinctions are not clear-cut. The concept that von Willebrand's disease may be a spectrum of laboratory and clinical variations has been suggested.1 Egeberg2 reported a family in which classic hemophilia seemed to be associated with features of von Willebrand's disease. Edson3 reported a number of families in which there was x-linked transmission of combined factor VIII and IX deficiency. In both these investigations, when platelet adhesiveness and bleeding time determinations were performed, abnormalities were frequently found; however, no platelet aggregation or release studies were presented.

Three patients with mild hemophilia (or the carrier state), probably inherited as a sex-linked trait, and an intrinsic platelet defect are presented below.
Table 1. Summary of Family History—Symptoms

<table>
<thead>
<tr>
<th>Family 1</th>
<th>Easy Bruising</th>
<th>Hemarthroses</th>
<th>Menorrhagia</th>
<th>Bleeding After Dental Extraction</th>
<th>Bleeding at Operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.D. (father)</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>E.D. (daughter)</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Has never been challenged</td>
</tr>
</tbody>
</table>

Family 2

P.S. (half sister)  Yes No Yes Yes Has never been challenged
W.D. (half brother) No Yes Yes Has never been challenged

CASE REPORTS (Table 1)

Family 1

L.D., a 70-yr-old white male of Italian extraction, gave a history of life-long easy bruising. His first surgical procedure was a hemorrhoidectomy performed without complications in 1962. However, subsequently, seven teeth were extracted with profuse bleeding which required massive transfusions of whole blood, fresh-frozen plasma, and fibrinogen before hemorrhage was controlled. The bleeding was most pronounced several days after the procedure. Because of these complications, he was referred for evaluation.

E.D., a 34-yr-old unmarried nurse and daughter of L.D., has had frequent episodes of purpura since childhood and profuse menorrhagia since menarche. She has not had any major surgery. The patient was first studied in 1964, following excessive bleeding after dental extraction, at which time a factor VIII level of 34% was noted.

Family 2

P.S. is a 23-yr-old white female referred because of excessive bleeding following a dental scaling procedure. In the past she had had two dental extractions and tonsillectomy without difficulty. However, the patient had a life-long history of easy bruising and menorrhagia since menarche. She has not undergone major surgical procedures. The family history was significant in that the maternal grandfather and uncle were “bleeders” and her half brother (W.D., below) has classical hemophilia. Her father and another brother (H.S.) were studied and found to have normal bleeding time, platelet adhesiveness, prothrombin time (PT), and activated partial thromboplastin time (PTT). The mother has not been available for study.

W.D., 27-yr-old half brother of P.S. through a common mother, was diagnosed as a hemophiliac during the first year of life because of excessive bleeding after a cut. He has had numerous episodes of hemarthrosis, as well as excessive bleeding following dental extraction. He has not had petechiae. Hemorrhage has always been controlled by fresh whole blood or fresh frozen plasma.

MATERIALS AND METHODS

Platelet-rich plasma (PRP) was obtained as follows: Nine volumes of human blood were collected into a tube containing 1 volume 3.8% sodium citrate and centrifuged at 23°C for 10 min at 50 g. Supernatant PRP was then removed by aspiration and used for the studies. Whole blood and plasma were exposed only to plastic surfaces. The platelet count varied from 200,000 to 350,000 per μl during these studies.

Platelet-poor plasma (PPP) was obtained as follows: Blood was collected as PRP (above) and centrifuged at 2500 g for 10 min. The platelet count was less than 40,000 platelets per μl.

Platelet aggregation (method of Born4) was performed in the following ways: One-half milliliter PRP was pipetted into a siliconized glass tube, diameter 8 mm, and stirred at a constant speed of 1200 rpm at 37°C. By means of a light source and a photoelectric cell, the aggregometer detects
changes in the transmission of light of the PRP as aggregation occurs. The aggregometer was set so that the per cent of transmittance of PRP was recorded as 0 and that of PPP 100. After addition of the aggregating agent, the changes in per cent transmission were continuously recorded as a function of time by a Heath Servo-Recorder, Model EU-20B. The aggregation was followed, and the difference between 0 transmittance and maximum transmittance was tabulated. This change was defined as the amplitude of the response. ADP (Sigma, St. Louis, Mo.), epinephrine (Winthrop Labs, New York), and soluble calf skin collagen (Worthington, Freehold, N.J.) were used in the concentration indicated in Fig. 1. Platelet aggregation was also performed with ristocetin (Abbott, North Chicago, Ill.) with 1.2 and 1.5 mg/ml.5

Standardized Ivy bleeding time was performed by the template method.6 Platelet retention was measured as described by Salzman7 using glass-bead columns obtained from Becton and Dickenson. Blood was drawn over the beads for a period of 45 sec. These columns were compared to homemade ones prepared by the method of Salzman7 and found to have comparable results.

Release of platelet-bound 14C serotonin was studied in PRP by the method of Harada and Zucker.8 Platelet antiheparin activity (platelet Factor 4) was performed by the procedure of Harada and Zucker.8 Since this parameter appeared to be log normally distributed, a 95% range is substituted for the standard deviation.

Clot retraction was performed by the method of Benthaus.9 Normal values were greater than 50% in 2 hr at 37°C.

Platelet factor 3 activity was performed by the method of Rabiner and Hrodek.10 Prothrombin time (PT) was performed by the procedure of Quick,11 using Simplastin (Warner-Chilcott, Morris Plains, N.J.) as the thromboplastin and a fibrometer (BioQuest, Cockeysville, Md.) to measure the clotting time.

Activated partial thromboplastin time (PTT) was performed by the procedure of Colman et al.12 Factor VIII coagulant activity was measured by the method of Hardisty and MacPherson.13 Test for factor VIII inhibitor was performed by the method of Robboy et al.14 Immunologic factor VIII activity was performed by the radioimmunoassay method of Hoyer15 through the courtesy of Dr. Leon Hoyer.

Gel filtration of PRP16 was carried out in a 3.3 x 5.5-cm plastic column packed with Sepharose 2B and washed with 400 ml 0.15 M Tris-HCl buffer, pH 7.4. A piece of Whatman No. 45 filter paper was positioned on the top of the column, and 10 ml PRP was added after the column had been equilibrated. One-milliliter fractions were collected in plastic tubes. The platelets appeared at the void volume.

Platelets for infusion were obtained as follows: Fresh whole blood collected in ACD was centrifuged at 50 g for 5 min to obtain platelet-rich plasma, which was aspirated under sterile conditions and then centrifuged at 2600 g for 5 min. Most of the platelet-poor plasma was removed, leaving the platelets in a final volume of 10-15 ml. This preparation was stored at 4°C and used for infusion within 24 hr of collection. One unit of platelets was the amount obtained from 450 ml blood.

Cryoprecipitate was obtained as follows: Fresh frozen plasma was thawed at 4°C for 24 hr and then centrifuged at 2600 g for 5 min. Cryoprecipitate from 4 U obtained from a single donor was pooled in a bag to a total volume of 25-50 ml and stored at -30°C.

RESULTS

Coagulation Studies (Table 2)

All four patients had normal prothrombin times. Patient L.D. had consistently prolonged activated partial thromboplastin times, whereas E.D. and P.S. had borderline elevated levels. All three had low factor VIII clotting activity. Patient W.D. did not have a factor VIII assay, but did have a prolonged PTT corrected by the addition of factor VIII but not by factor IX. No evidence for a factor VIII inhibitor14 was found in L.D., E.D., or P.S.

Immunologic factor VIII. Despite repeatedly low factor VIII by clotting
Table 2. Plasma Coagulation System

<table>
<thead>
<tr>
<th>Patient</th>
<th>Prothrombin Time (Sec)</th>
<th>Partial Thromboplastin Time (Sec)</th>
<th>Factor VIII Clotting Activity (% normal)</th>
<th>Factor VIII Immunoassay (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. D.</td>
<td>12.5*</td>
<td>43.1</td>
<td>7.5</td>
<td>223</td>
</tr>
<tr>
<td>E. D.</td>
<td>13.0</td>
<td>35.9</td>
<td>29</td>
<td>200</td>
</tr>
<tr>
<td>P. S.</td>
<td>12.3</td>
<td>36.2</td>
<td>33</td>
<td>209</td>
</tr>
<tr>
<td>W. D.</td>
<td>13.0</td>
<td>Prolonged†</td>
<td>Low†</td>
<td>Unavailable</td>
</tr>
<tr>
<td>Normal</td>
<td>12.5 ± 1.5§</td>
<td>29.5 ± 7.5§</td>
<td>50–200</td>
<td>80–160</td>
</tr>
<tr>
<td>Hemophilia</td>
<td>Normal</td>
<td>Prolonged</td>
<td>0–50</td>
<td>100–250</td>
</tr>
<tr>
<td>von Willebrand</td>
<td>Normal</td>
<td>Prolonged</td>
<td>Usually &lt; 50</td>
<td>**</td>
</tr>
</tbody>
</table>

*All determinations are means of three separate determinations on different days.
†Sixty-eight seconds with control of 41 sec (done at Baylor College of Medicine).
§Patient 71 sec with control of 45 sec in substitution PTT. Patient's clotting time was corrected to that of control with factor VIII but not with factor IX (done at Baylor College of Medicine).
§§Means ± 2 SD.
¶95% range.
§ Some patients with von Willebrand's disease have normal factor VIII clotting activity.
**Proportional to factor VIII clotting activity.

assay, patients L.D., E.D., and P.S. all had significantly greater than normal immunologic factor VIII (Table 2).

Platelet function studies (Table 3). All patients had normal platelet counts and had not taken any medication for more than 10 days. Platelet size and appearance estimated on Wright-stained peripheral blood smears were normal in all patients. All patients, with the exception of W.D., had prolonged bleeding times on every occasion tested. E.D. was mildly prolonged. In contrast, W.D. had a normal bleeding time. Platelet adhesiveness was also uniformly abnormal on multiple tests in patients L.D., E.D., and P.S. (it was not assayed in W.D.). Platelet factor 3 activity and clot retraction were normal in all four patients.

Platelet aggregation (Fig. 1). Only patients L.D., E.D., and P.S. were available for these studies, and all showed significantly less than normal aggregation response to ADP, epinephrine, and collagen. No second wave was observed. On the other hand, platelets from patients P.S., E.D., and L.D. showed 100% response to ristocetin.

Platelet antiheparin factor release (Table 4). All patients showed abnormal release of platelet antiheparin activity when exposed to epinephrine and collagen. Only patient E.D. was abnormal with ADP, but patients L.D. and P.S. were at the low end of the 95% range.

Table 3. Platelet Function

<table>
<thead>
<tr>
<th>Patient</th>
<th>Bleeding* Time (min)</th>
<th>Platelet Count* (per μl)</th>
<th>Platelet Retention (%)</th>
<th>Platelet Factor 3 (Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.D.</td>
<td>13</td>
<td>172,000</td>
<td>6</td>
<td>35</td>
</tr>
<tr>
<td>E.D.</td>
<td>9.5</td>
<td>257,000</td>
<td>8</td>
<td>35</td>
</tr>
<tr>
<td>P.S.</td>
<td>17</td>
<td>215,000</td>
<td>0</td>
<td>57</td>
</tr>
<tr>
<td>W.D.</td>
<td>5.6</td>
<td>296,000</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Normal</td>
<td>6 ± 1</td>
<td>250,000 ± 50,000</td>
<td>32 ± 10†</td>
<td>49 ± 10†</td>
</tr>
</tbody>
</table>

*All values are means of three determinations except for W.D.
†Means ± 1 SD for 20 normal subjects.
Platelet 

Platelet release (Table 4). Patient L.D. showed normal release of 

Study of gel-filtered platelets. Platelets from P.S., E.D., and L.D., as well as normal platelets, were studied after gel filtration. 0.3 ml platelets suspended in buffer were added to 0.2 ml normal PPP and studied in the aggregometer. When patients' gel-filtered platelets were suspended in normal PPP, second-phase aggregation was absent. On the other hand, when normal platelets were suspended in PPP of P.S., L.D., and E.D., second wave aggregation was normal with ADP, epinephrine, and collagen. Platelet counts of aggregation mixture ranged from 100,000 to 200,000 per microliter.

Effect of therapy with prednisone. Prior to an elective dental procedure, patient P.S. was given 60 mg prednisone in three divided doses for 3 days. Bleeding times and platelet adhesiveness were determined prior to and at the end of the trial. There was no improvement of any of the parameters studied.

Effect of cryoprecipitate and plasma infusion. Patient L.D. was studied 1, 4, 6, 24, and 30 hr after receiving an infusion of 8 U of cryoprecipitate.

Table 4. Platelet-release Function

<table>
<thead>
<tr>
<th>Patient</th>
<th>¹⁴C Serotonin Release (%)</th>
<th>Platelet Antiheparin Factor Release U/10¹¹ Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ADP</td>
<td>Epinephrine</td>
</tr>
<tr>
<td>L.D.</td>
<td>52</td>
<td>54</td>
</tr>
<tr>
<td>E.D.</td>
<td>5.1</td>
<td>40</td>
</tr>
<tr>
<td>P.S.</td>
<td>1.6</td>
<td>3.5</td>
</tr>
<tr>
<td>Normal</td>
<td>50 ± 10*</td>
<td>64 ± 10*</td>
</tr>
</tbody>
</table>

*Means ± 1 SD.
†95% range.
Factor VIII clotting activity rose from a baseline of 7.5% to 100% of normal 1 hr postinfusion and returned to a value similar to the patient’s original factor VIII level at 30 hr. Bleeding time shortened from baseline of 13 min to 8 min at 1 hr postinfusion; it was 8½ min at 24 hr postinfusion and had lengthened to 15½ min by 36 hr. Platelet aggregation and platelet antiheparin-factor release did not significantly improve at 1 or 24 hr postinfusion.

Patient E.D. was studied after infusion of 2 U fresh-frozen plasma. Prior to infusion, factor VIII clotting level was 34%. Following infusion of 2 U of fresh-frozen plasma, level rose to 60% (calculated rise 59%). By 4 hr the level was 40%, and the level returned to baseline at 24 hr.

**Effect of platelet infusion.** In a separate experiment, 8 U of platelets were infused in patient L.D. Four hours later, bleeding time remained prolonged at 15.5 min, and platelet adhesiveness was 0%. However, 24 hr following platelet infusion, bleeding time shortened to 10.5 min, and platelet adhesiveness was 17%. Although somewhat corrected, the values were still abnormal. Platelet aggregation and platelet antiheparin-factor release after exposure to ADP, epinephrine, and collagen did not improve at 4 or 24 hr after platelet transfusion.

**DISCUSSION**

In two families investigated, three of the four individuals had the triad of findings usually associated with von Willebrand’s disease, i.e., low factor VIII, long bleeding time, and low platelet adhesiveness. Further evaluation, however, revealed abnormal platelet aggregation with ADP, epinephrine, and collagen. This finding is not present in von Willebrand’s disease and suggests that the cause of the abnormal bleeding time resides in platelets rather than in plasma. This hypothesis is supported by the observation that platelets from the three patients remained abnormal when suspended in normal plasma and that normal platelets functioned normally in patients’ plasma.

Patients with defective secondary aggregation have been reported by several observers in 1967. Since that time, similar patients have been studied in more detail and have been found to have abnormal release of certain intracellular components such as ADP, 5-hydroxytryptamine, and platelet factor 4 (antiheparin activity). Some of the patients also gave evidence of abnormal availability of platelet factor 3. Studies by Holmsen and Day have demonstrated two pools of nucleotides in platelets, a metabolic pool and a storage pool localized in the dense granules and liberated during the release reaction. Some patients with platelet release abnormality have been shown to have a deficiency of the stored components and have markedly decreased dense bodies, a condition termed storage pool disease. Other patients were shown to have normal storage pools of adenine nucleotides, but presumably have an abnormal release mechanism. The release reaction in our patients was quantified by measuring the release of platelet antiheparin activity (platelet factor 4) and ¹⁴C serotonin. Our patients showed abnormalities in both. Storage pool disease could not be distinguished from an abnormality in the release mechanism.

What makes these patients unique is the associated low factor VIII levels observed on repeated occasions. Patients L.D. and E.D., a father and daughter,
had factor VIII clotting activities of 7.5% and 29%, respectively. Based on these values, the father probably has mild hemophilia, and the daughter carries the trait. Although consistent with an X-linked trait, the family history does not rule out dominant inheritance. Patient P.S. had an average value of 35% factor VIII. Her half-brother W.D., who was studied at another institution, had a normal Ivy bleeding time on several occasions. Unfortunately, platelet function assessment could not be performed. A life-long history of bleeding episodes with hemarthrosis and the laboratory abnormalities of long clotting time, low factor VIII clotting activity, and normal factor IX are consistent with the diagnosis of classical hemophilia. The report that the maternal grandfather and great uncle were “bleeders” is consistent with a family pedigree of sex-linked recessive transmission as is seen in hemophilia.

The initial laboratory findings in both these families might be explained by a platelet-release abnormality coinciding with either classical hemophilia or von Willebrand’s disease. Five female members of such a family with a dominant inherited bleeding disorder have been studied by Crowell and Eisner.25 That family differs from those in this investigation in that they also manifested mild thrombocytopenia, large platelets, and low platelet factor 3. Although the authors concluded that this was an overlap of von Willebrand’s disease and a “hereditary thrombopathy,” no studies were performed to distinguish von Willebrand’s disease from hemophilia. Zimmerman et al. have recently reported differentiation of classical hemophilia and von Willebrand’s disease26 and detection of the carrier state27 for hemophilia by an immunologic technique. Despite low clotting activity of factor VIII, our three patients studied had greater than 200% of the normal immunologically reactive material, a finding associated with classical hemophilia or its carrier state. If von Willebrand’s disease were present, the immunologically reactive material would have been at a low level similar to that of the coagulant factor VIII activity. Aggregation with ristocetin5,28 has been found to be abnormal in von Willebrand’s disease; hence the normal ristocetin-induced aggregation in patients L.D., E.D., and P.S. is another point against the diagnosis of von Willebrand’s disease. Another method of differentiating classic hemophilia from von Willebrand’s disease is the response to infusion of fresh-frozen plasma or cryoprecipitate. Patient L.D., who was studied after receiving an infusion of cryoprecipitate, showed the expected rise in factor VIII activity followed by the rate of disappearance of factor VIII activity associated with classic hemophilia, rather than the sustained elevation observed in true von Willebrand’s disease.29 Similar results were observed in his daughter. Platelet adhesion, aggregation, and antiheparin-activity release did not improve at 1 or 24 hr postinfusion, suggesting that the defects were not due to the deficiency of a plasma factor. Nevertheless, the patient’s bleeding time shortened following cryoprecipitate infusion. Cryoprecipitate has been noted to cause return of bleeding time to normal temporarily in normal patients who are taking aspirin.30,31 Alternatively, one might explain this in the following manner: The patient has two defects, one in the platelets, or primary hemostatic mechanism, and the other in the intrinsic system, or secondary hemostatic mechanism. The defect in the secondary mechanism, which, if it were the sole defect, would not result in an abnormal bleeding time, accentuates
the long bleeding time associated with the defect in the primary mechanism. This situation is analogous to that seen in a hemophiliac taking aspirin. When the secondary defect is corrected with infusion of cryoprecipitate, the bleeding time will reflect the platelet-defect effectiveness of the platelet alone.

An infusion of platelets given to L.D. did not significantly alter the bleeding time measured at 4 hr but shortened it at 24 hr. This finding may be analogous to data indicating that platelet function improves after the platelets stored at 22°C have been in vivo for 24 hr. Whether platelets behave the same in vivo after storage at 4°C, as in our case, is not clear yet. There was no evidence of improvement of platelet function as measured by aggregation or release of anti-heparin activity. However, this might be accounted for by the relatively smaller number of "normal" platelets compared to the patient’s own abnormal platelets. The bleeding time may be a more sensitive measure of the in vivo effectiveness of platelets.

These cases probably represent the occurrence of two relatively common congenital hemorrhagic diseases in the same patient. The three patients who appeared initially to have von Willebrand’s disease had, in fact, mild hemophilia, or the carrier state, and a platelet-release abnormality. W.D., on the other hand, seems to be a classic hemophiliac with no platelet abnormality. Table 5 summarizes the values of the PTT, factor VIII (clotting and immunologic activity), and platelet-function tests seen in von Willebrand’s disease, three qualitative platelet disorders, classic hemophilia, and in our three patients. The pattern of laboratory abnormalities in our patients does not coincide with any single disease, but is consistent with two defects—platelet release abnormality and classic hemophilia.

Since the screening tests performed routinely in many coagulation laboratories may be misleading in such cases, suggesting von Willebrand’s disease alone, it is recommended that, in order to establish a definite diagnosis of von Willebrand’s disease, platelet aggregation is a valuable diagnostic criterion along with the triad of low factor VIII, long bleeding time, and low platelet adhesiveness. Immunologic assays of factor VIII are also of value in dis-

<table>
<thead>
<tr>
<th></th>
<th>Plasma Coagulation System</th>
<th>Platelet Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>von Willebrand’s syndrome</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Bernard-Soulier syndrome</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Thrombasthenia</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Platelet-release abnormality</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Hemophilia</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Present patients</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

*Not performed.
†Colman and Hoyer, unpublished results.
Plus indicates increase; minus indicates decrease.
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tinguishing von Willebrand's disease from hemophilia. If these tests cannot be performed or if they do not confirm the presence of true von Willebrand's disease, assessment of the response to cryoprecipitate or plasma may be crucial, since the treatment of von Willebrand's disease is different from that in the syndrome described in this communication. Optimal management of the patients described here may require both platelets and fresh-frozen plasma or cryoprecipitate in order to prevent bleeding during or after surgery.

REFERENCES

2. Egeberg O: Changes in the activity of antihemophilic A factor (factor VIII) and in the bleeding time associated with muscular exercise and adrenal infusion. Scand J Clin Lab Invest 15:539, 1963
26. Zimmerman TS, Ratnoff OD, Powell...
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