The Bleeding Time is Longer Than Normal in Hemophilia

By M. Elaine Eyster, Robert A. Gordon, and James O. Ballard

Bleeding times were performed on 71 hemophiliacs using the Simplate II device. Eight patients receiving Indocin or Motrin for hemophilic arthropathy were evaluated separately from the remaining 63 who had a mean bleeding time of 7.65 ± 3.20 min (1 SD) compared to the control group of 5.35 ± 1.49 min (p < 0.005). No difference was found when 26 mild hemophiliacs who had received <10,000 U of clotting factor concentrate the previous year and no infusions in at least 3 mo were compared with 28 severe hemophiliacs who had received >20,000 U of clotting factor concentrate the previous year and had been infused within 1 mo of testing. Ten patients (16%) had bleeding times greater than 10 min. Bleeding times remained prolonged on repeat evaluations in 7 of these patients. 3 of whom had mild disease and all of whom had normal platelet aggregations in response to arachidonic acid. We conclude that the bleeding time is longer than normal in hemophilia. This abnormality is not related to disease severity, recent transfusions, or the use of nonsteroidal antiinflammatory drugs.

HEMOSTASIS is defined as the spontaneous arrest of bleeding from ruptured vessels. With minimum injury involving only a few endothelial cells from capillaries, small venules, or arterioles, the defect is effectively sealed by the formation of the platelet plug. This process, termed primary hemostasis, is measured in vivo by the bleeding time and is said to be normal in hemophilia.1, 4

While reviewing data collected by the Hemophilia Study Group, we noticed that 32 of 376 patients (8.5%) had bleeding times recorded at the upper limit of normal or above. This finding prompted us to study the bleeding times of 71 hemophiliacs attending our clinic. We found that the mean bleeding time of hemophiliacs was significantly longer than normal controls. This difference was not related to disease severity, recent transfusions, or the use of nonsteroidal antiinflammatory drugs.

MATERIALS AND METHODS

Seventy-four (74) patients ranging from 4 to 68 yr of age (mean 23 yr) were studied. Seventy-one had classic hemophilia and 3 had Christmas disease (factor IX deficiency). Thirty-seven had factor VIII or IX levels equal to or less than 0.01 µ/ml, 10 had levels >0.01 but <0.05 µ/ml, 13 had levels 0.05-0.10 µ/ml, and 11 were >0.10 µ/ml. All patients had normal ristocetin cofactor activities and normal factor-VIII-related antigens. Bleeding times were performed at regularly scheduled clinic visits when detailed drug histories were taken, liver function studies were obtained, and transfusion records were reviewed. Three patients who had taken aspirin were excluded, leaving 71 evaluable patients.

Template bleeding times were performed with informed consent using the Simplate II device (General Diagnostics, Morris Plains, N.J.). A blood pressure cuff was inflated to 40 mm Hg pressure, and two incisions 6 mm in length and 1 mm in depth were made parallel to the antecubital crease on the volar surface of the forearm in an area free from large vessels. Results were expressed as the average bleeding time of the two incisions. All tests were performed by one of four specially trained technologists. Forty healthy males between the ages of 18 and 47 yr served as controls. Bleeding times with transverse incisions (parallel to the antecubital crease) were performed on one group of 20 by one of the technicians who had tested the study group. Bleeding times on another group of 20 were performed with vertical incisions by one of the authors, and the results of the two methods compared. Bleeding times were considered to be “normal” if they fell within the range of values obtained in either control group (2.5-8 min).

Factor VIII coagulant activity (VIII:C) was determined on all patients by a one-stage assay based on the activated partial thromboplastin time (aPTT), as previously described, except that Kaolin and Thrombofax (Ortho Diagnostics, Raritan, N.J.) were used as activator and phospholipid.5 Factor-VIII-related antigen (VIII:R:Ag) was performed by the Laurell technique of quantitative immunoelectrophoresis, and the von Willebrand factor (VIII:R:WF) was measured by a modification of the washed platelet ristocetin assay6 as previously described.7 Two-dimensional crossed immunoelectrophoresis of the FVIII:R:Ag was performed on selected patients, as previously described, by the technique of Laurell.8 Platelet aggregation studies using platelet-rich plasma were performed with a Chrono-log aggregometer on citrated platelet-rich plasma that had been centrifuged at 150 g (800 rpm) at 25°C for 6 min, with the platelet count adjusted to approximately 200,000 cu/mm. The aggregating agents used, expressed as final concentrations, included ADP (3 µM), epinephrine (48 µg/ml), collagen suspension (190 µg/ml), ristocetin (1.4 mg/ml), bovine thrombin (0.12 U/ml), and arachidonic acid reconstituted in Tris buffer, (220 µg/ml). Platelet suspensions washed with Krebs Henseleit buffer were tested with arachidonic acid (1 µg/25 µl).

Statistical Methods

The level of significance (p) of the differences between the two arithmetic means was calculated using the Student's t test.9

RESULTS

Eight patients who were receiving indomethacin (Indocin) or ibuprofen (Motrin) for hemophili
arthropathy were evaluated separately from the remaining 63 patients who had a bleeding time of 7.65 ± 3.20 min (Fig. 1). When compared with the individual control groups, the hemophiliacs had a longer mean bleeding time than controls with transverse incisions ($p < 0.005$) or with vertical incisions ($p < 0.001$). The mean bleeding time for controls with transverse incisions was 5.35 ± 1.49 compared to 4.38 ± 1.07 for those with vertical incisions ($p < 0.025$ but >0.0125). Ten of 63 patients (16%) had bleeding times greater than 10 min, while none of the 40 control subjects had a bleeding time greater than 8 min. No difference was found when 26 mild hemophiliacs who had received <10,000 U of clotting factor concentrate the previous year and no infusions in at least 3 mo were compared with 28 severe hemophiliacs who had received >20,000 U of clotting factor concentrate the previous year and had been infused within 1 mo of testing. There were no relationship between age and bleeding time (data not shown).

Nine patients with bleeding times greater than 10 min were studied in more detail (Table 1). Bleeding times remained abnormal in 7 of the 9 on repeat determinations. Two patients had family histories of sex-linked hemophilia. Seven had no affected relatives. Von Willebrand’s disease was excluded on all by measurement of the VIIIR:WF and by two-dimensional electrophoresis into agar containing FVIII antibody. All had normal serum alanine transferases. Platelet aggregations were normal on 6 of 8 patients tested, and the two with abnormal responses had normal responses when washed platelets were tested with arachidonic acid.

**DISCUSSION**

The bleeding time has been defined as the time between the infliction of a small standard cut and the moment when bleeding stops. Two mechanisms are involved in the formation of the platelet plug that seals the ends of the severed vessels. The first, which is dependent on the plasma von Willebrand factor, involves the interaction of platelets with collagen and subsequent degranulation with release of ADP. The second involves platelet aggregation in the presence of fibrinogen in response to ADP and traces of thrombin produced at the site of vessel damage. The bleeding time is said to be normal in hemophilia because platelet adhesion and aggregation in response to ADP are normal, and because the mechanism that is initiated by tissue factor in the absence of factor VIII coagulant activity can apparently generate enough thrombin to stop bleeding from the small injury administered by the test.

In 1910, Duke clearly showed that the prolonged bleeding time was related to the platelet count rather than to the coagulation time. He found the bleeding time to be normal in hemophilia and in most other coagulation abnormalities with the exception of hypo-fibrinogenemia and some cases of factor V deficiency. Borchgrevink in 1961 reported that the bleeding time was normal in all 14 patients with severe hemophilia studied, as well as in patients with other congenital deficiencies, with the exception of von Willebrand’s disease. Kaneshiro in 1969 confirmed that the mean bleeding time was normal in hemophilia. However, 2 of 11 patients with severe hemophilia A had prolonged baseline bleeding times of 12 and 15 min, respectively.

In our study of 63 hemophiliacs who were not receiving nonsteroidal antiinflammatory drugs, the mean bleeding time of 7.65 ± 3.20 min was significantly longer than the control group of 5.35 ± 1.49 studied by comparable methods ($p < 0.005$). Ten patients had bleeding times over 10 min, with the normal range being 2.5–8 min.

Hathaway et al. have shown that cryoprecipitate and factor VIII concentrate may cause prolonged bleeding times and paradoxical bleeding in intensively transfused patients. These abnormalities were related either to abnormal platelet function or to increased levels of circulating fibrin monomer, fibrin-split products, or fibrinogen interfering with the thrombin–fibrinogen reaction. Fibrin monomer or fibrin split products were not measured in our patients. However, the abnormalities we described cannot be attributed to transfusion, since no difference was found in the mean bleeding times of 28 patients who had been intensively transfused and those who had received no infusions in over 3 mo.

A number of investigators have shown that aspirin increases the bleeding time in normal individuals and in hemophiliacs. Kaneshiro et al. showed that 10 of 19 hemophiliacs had markedly prolonged bleeding...
Table 1. Clinical and Laboratory Data on Patients With Prolonged Bleeding Times

<table>
<thead>
<tr>
<th>Patient</th>
<th>Bleeding Time (2.5–8 mm)</th>
<th>Platelet Count (200–400 x 10^3/cu mm)</th>
<th>Platelet Aggregation</th>
<th>VIII:C 0.5–1.5 μl/ml</th>
<th>VIII:RAg 0.5–2.0 μl/ml</th>
<th>VIII:WF 0.4–1.2 μl/ml</th>
<th>Crossed Immunoelectrophoresis</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>W.S. 11</td>
<td>17, 11.5, 10.5</td>
<td>285</td>
<td>ND</td>
<td>.06</td>
<td>1.05</td>
<td>.78</td>
<td>NL</td>
<td>Infrequent bleeding episodes. No infusions &gt; 5 mos. Platelet factor 3 normal. SGOT 53 units</td>
</tr>
<tr>
<td>M.C. 6</td>
<td>13, 9</td>
<td>“Normal” on smear</td>
<td>ND</td>
<td>.01</td>
<td>2.27</td>
<td>1.21</td>
<td>NL</td>
<td>Infrequent bleeding episodes. No infusions &gt; 5 mos. SGOT 29 units. FVIII inhibitor. FIX infusion day preceding aggregation study. Av. 130,000 U FIX previous year SGOT 38 units</td>
</tr>
<tr>
<td>B.M. 7</td>
<td>15, 6</td>
<td>162</td>
<td>ND</td>
<td>.01</td>
<td>1.51</td>
<td>.71</td>
<td>NL</td>
<td>Infrequent bleeding episodes. No infusions &gt; 5 mos. SGOT 29 units. FVIII inhibitor. FIX infusion day preceding aggregation study. Av. 130,000 U FIX previous year SGOT 38 units</td>
</tr>
<tr>
<td>V.G. 7</td>
<td>11.5, 12.5</td>
<td>325</td>
<td>ND</td>
<td>.13</td>
<td>1.41</td>
<td>.81</td>
<td>NL</td>
<td>No infusions &gt; 3 mos; total 4 in lifetime (cryo only). Family history of sex-linked hemophilia. SGOT 6 units</td>
</tr>
<tr>
<td>M.S. 27</td>
<td>&gt; 17*, 11</td>
<td>101</td>
<td>ND</td>
<td>.01</td>
<td>1.74</td>
<td>.75</td>
<td>NL</td>
<td>“Prophylaxis” b.i.w. with FVIII. Av. 70,000 U yearly. SGOT 46 units</td>
</tr>
<tr>
<td>D.D. 12</td>
<td>&gt; 18, 14, 16</td>
<td>132</td>
<td>ND</td>
<td>.01</td>
<td>1.3</td>
<td>.65</td>
<td>NL</td>
<td>On FVIII q.d. plus prednisone for synovitis of knee. Av. 57,000 U yearly. SGOT 43 units</td>
</tr>
<tr>
<td>J.Y. 27</td>
<td>&gt; 17*, 10, 10</td>
<td>“Normal” on smear</td>
<td>ND</td>
<td>.02</td>
<td>2.19</td>
<td>1.12</td>
<td>NL</td>
<td>Also has Tha minor. No infusions in 1 yr. SGOT 32 units</td>
</tr>
<tr>
<td>S.C. 27</td>
<td>&gt; 17*, 15</td>
<td>119</td>
<td>ND</td>
<td>.10</td>
<td>0.96</td>
<td>0.83</td>
<td>NL</td>
<td>No infusions for 2 yr. Total 25 bags cryo in lifetime. Family history of sex-linked hemophilia. SGOT 15 units</td>
</tr>
<tr>
<td>M.A. 19</td>
<td>&gt; 17*, 7</td>
<td>“Normal” on smear</td>
<td>ND</td>
<td>&lt;.01</td>
<td>2.03</td>
<td>.59</td>
<td>NL</td>
<td>Severe hemophilia. Received 44,000 U FVIII during years studied. SGOT 48 units</td>
</tr>
</tbody>
</table>

Abbreviations: PIP, platelet-rich plasma; Pits, platelets; AA, arachidonic acid; NL, normal; ND, not done.

*Receiving Indocin (IMS, JY, MA) or Dristan (SC).
†More than 50% change in light transmission induced by ADP, collagen, ristocetin, thrombin, or arachidonic acid, and the appearance of a second wave with epinephrine were considered normal.

*times after aspirin, with 8 patients still bleeding briskly when the test was terminated after 40 min. In a subsequent study by Kasper et al., differences in the degree of prolongation of the bleeding time by aspirin were thought to result from a consistent difference between individual patients rather than from a different response of the same patient on different occasions. The reason for the variation was unknown, since all patients had equally impaired aggregation with collagen and no second wave of aggregation with epinephrine.

Careful drug histories obtained at the time the bleeding times were performed were felt to reliably exclude aspirin and other nonsteroidal inflammatory drugs known to impair platelet function as a cause of the prolonged bleeding times in our patients. In addition, when 9 of our 10 patients with bleeding times greater than 10 min were studied in more detail, all but 2 were found to have normal platelet function. Moreover, the two with mild abnormalities had normal aggregation to arachidonic acid when washed platelets were used, indicating that the conversion of arachidonic acid to thromboxane A₂ was not impaired as would be expected if either patient had taken
aspirin or other drugs that interfere with prostaglandin metabolism. This finding, although unexplained, suggested that these two patients may have had a release defect for arachidonic acid, possibly mediated by a plasma factor.

Liver disease has been reported to cause a prolonged bleeding time. However, all of our patients with prolonged bleeding times had normal or near normal serum alanine transferases, making this possibility unlikely.

The question as to why the bleeding time is longer in hemophiliacs than in normal males remains unanswered. Since our initial report, Buchanan and Holtkamp have demonstrated a prolonged bleeding time in 10 of 49 hemophiliacs who had not received transfusions in at least 72 hr. None of these patients had evidence of von Willebrand’s disease or prior aspirin ingestion. In Buchanan’s study, as well as in ours, there was a suggestion of bimodality, which could represent a subset of hemophiliacs with an unexplained and as yet unidentified defect in primary hemostasis.

Although standardization of the bleeding time is difficult, it is unlikely that variables such as differences in technique related to the site of skin puncture, vascularity, or direction or depth of incision, account for the marked prolongation of the bleeding time in 16% of our patients and 20% of Buchanan’s patients. Some have claimed that bleeding times are longer with transverse than with vertical incisions. However, published data do not support this statement, and Bowie et al. have found that, of 19 tests on 16 normal individuals, there was no significant difference between transverse and longitudinal incisions, except that blood loss was higher after longitudinal cuts. Although our control group with transverse incisions had slightly longer bleeding times than those with vertical incisions, no control subject’s bleeding time in either group exceeded 8 min, and the mean bleeding times of both groups of 20 performed by different individuals were significantly different from our study population. Furthermore, if the abnormalities described were due to technique, one would not expect to find results of greater than 10 min repeatedly on the same individuals.

If the prolongation were due to the delayed rate of thrombin generation and fibrin formation affecting platelet plug formation in patients with severe disease, one would not expect to find this abnormality in mildly affected hemophiliacs with factor VIII levels of 0.06–0.13 μ/ml, as was noted in three of our patients in whom von Willebrand’s disease had been excluded by quantitative as well as qualitative studies of the factor-VIII-related antigen and by measurement of the ristocetin cofactor. Therefore, it seems most likely that the prolonged bleeding time is due to a separate but interrelated abnormality that has yet to be defined.

Whatever the reason, the bleeding time is not always normal in hemophilia. Patients with factor VIII procoagulant deficiency and a prolonged bleeding time do not necessarily have von Willebrand’s disease or hemophilia with a coexistent platelet functional abnormality. This observation has important diagnostic implications.

REFERENCES


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ME Eyster, RA Gordon and JO Ballard