Thrombocytopenia in Septicemia: The Role of Disseminated Intravascular Coagulation

By Peter B. Neame, John G. Kelton, Irwin R. Walker, Iain O. Stewart, Hymie L. Nossel, and Jack Hirsh

The mechanism of isolated thrombocytopenia in sepsis is unknown, but compensated disseminated intravascular coagulation (DIC) has been suggested as a possible cause. To investigate this possibility, platelet counts and sensitive assays for in vivo thrombin and plasmin generation, including fibrinogen gel chromatography and fibrinopeptide A (FPA) assays, were obtained on 31 septicemic patients. Fifteen of 17 patients with gram-negative sepsis and 8 of 14 patients with gram-positive sepsis had thrombocytopenia. Platelet survival studies demonstrated a decreased platelet survival. In 11 of 12 patients with severe thrombocytopenia (platelet count <50,000/µl), there was laboratory evidence of intravascular coagulation. In contrast, there was little evidence of intravascular coagulation in 8 of 11 patients with moderate thrombocytopenia (platelet counts 50,000 to <150,000/µl) or in 7 of 8 patients with normal platelet counts. This report indicates that while DIC accompanies thrombocytopenia in many patients with severe thrombocytopenia, there is frequently little evidence for intravascular coagulation in patients with moderate thrombocytopenia. It is apparent that factors other than intravascular thrombin must play a role in producing the thrombocytopenia of sepsis.

Coagulation abnormalities, ranging from a rapidly fulminating state of disseminated intravascular coagulation (DIC) to a slight elevation in the level of fibrin/fibrinogen degradation products, are well recognized in patients with bacterial sepsis. Recent reports have emphasized that thrombocytopenia frequently occurs early in the course of sepsis without overt evidence of DIC in both adults and children. The mechanism responsible for this thrombocytopenia is not fully understood. While thrombocytopenia is almost invariably observed in septicemic patients with overt DIC, it may also occur in patients with septicemia without laboratory evidence of disseminated intravascular coagulation and, in these circumstances, it has been suggested that subclinical or compensated DIC might be a contributing factor.

We have performed a study to investigate the contribution of intravascular coagulation to the thrombocytopenia in sepsis. Serial platelet counts and sensitive tests of fibrinogen/fibrin proteolysis, including fibrinogen gel chromatography and fibrinopeptide A assays, were performed in 31 septicemic patients. The results are reported in this article.

Materials and Methods

Patients

Thirty-one patients (ages 23--91 yr, mean 63 yr; M:F = 18:13) with sepsis associated with a variety of infections, including pneumonia, septicemia, subacute bacterial endocarditis, acute cystitis, acute cholecystitis, and intraabdominal infection, were studied over a 36-mo period from December 1974 to December 1977. Patients were included in the study if they had a clinical diagnosis of sepsis that was verified by positive blood cultures. Patients with diseases known to cause thrombocytopenia or other coagulation abnormalities independently (for example, patients with severe liver disease, chronic alcoholism, leukemia) were excluded from the study.

Laboratory Investigations

Platelet studies. Platelet counts were performed using a Coulter ZB1 (Coulter Electronics). Platelet survival studies were performed using ¹¹¹I-labeled homologous platelets. The normal half-life (T½ ¹¹¹I) in our laboratory, determined from 20 normal subjects, is 80-120 hr.

Coagulation studies. The following coagulation assays were performed: plasma fibrinogen, factors V, VIII, and XII.

Methods for detecting thrombin and plasmin activation. The following tests were performed. (1) The fibrin/fibrinogen degradation products (FDP) were assayed by latex agglutination (Wellcome Reagents, General Diagnostics, Scarborough, Ontario, Canada). Blood was collected into acid citrate (9:1 v:v) and aminocaproic acid (EACA; 40 µl/ml). After centrifugation, 1000 µg bovine thrombin (Bovine Thrombin, Parke-Davis) and 0.1 M EACA plus 0.25 M CaCl₂ were added to the supernatant, and 30 min later, it was centrifuged. The serum was diluted in saline glycine buffer (pH 8.2), and equal volumes of latex suspension and sera were incubated together. The end-point for the assay was the last dilution that agglutinated the latex. The normal value in our laboratory, determined from 40 normal controls, is a titer of less than 1:8, corresponding to a level of fibrinogen/fibrin degradation products (FDP) of 24 µg/ml.

(2) Protamine sulfate paracoagulation test (PS) for fibrin was performed by the method of Kidder et al. Blood was collected as for FDP. Using 1% protamine sulfate (Eli Lilly Co.), a positive result was reported if visible fibrin strands formed at 30 min at 37°C, or at 24 hr at room temperature. The test result was negative in 50 normal controls.
THROMBOCYTOPENIA IN SEPSIS: ROLE OF DIC

(3) Fibrinogen gel chromatography (FGC) was performed on platelet-poor plasma by the method of Alkjaersig and Fletcher\textsuperscript{11} using an automated system (Sherwood Medical Research Development, St. Louis Mo.). The percentage of immunoreactive fibrinogen-derived protein circulating as high molecular weight fibrinogen/fibrin complexes in normal subjects is less than 0.5% of the fibrinogen level based on a study of 50 normal individuals in our laboratory.

(4) Fibrinopeptide A (FPA) assay was collected according to the method of Nossel et al.\textsuperscript{12} in a heparin/trasylol mixture (4.5 ml blood and 0.5 ml heparin [500 \( \mu \)l] and trasylol [500 \( \mu \)l]), transported deep frozen, and assayed in batches in Dr. Nossel’s laboratory at Columbia University, New York. The normal range of the FPA assay is 0–1.3 pmole/ml.

Statistical Methods

Differences between groups were analyzed by one-way analysis of variance and the Student-Newman Keuls test.\textsuperscript{11}

Platelet Studies

Table 1 shows the bacteria involved in the production of the septicemia, the associated disease, the antibiotics used, and the lowest platelet count observed in the patients. Serial platelet counts and coagulation studies were performed on 17 patients with gram-negative septicemia and 14 patients with gram-positive septicemia. Thrombocytopenia was defined as a platelet count less than 150,000/\( \mu l \). Fifteen of the 17 patients with gram-negative septicemia and 8 of 14 patients with gram-positive septicemia had thrombocytopenia. The platelet count ranged from 2000–145,000/\( \mu l \) in gram-negative and 10,000–80,000/\( \mu l \) in gram-positive patients.

Platelet survival studies were performed on three patients while they were thrombocytopenic and on two patients who were not thrombocytopenic. None of these five patients had received previous blood products nor had they been pregnant. The platelet T\( \text{1/2} \) in the two patients with normal platelet counts were at the lower limits of the normal range (78 and 82 hr). In contrast, the platelet T\( \text{1/2} \) was reduced in all three patients with thrombocytopenia and the patient with the lowest platelet count (25,000/\( \mu l \)) had the shortest platelet T\( \text{1/2} \) (29 hr) (Table 2).

Coagulation Assays

There was no statistical relationship between the platelet count and the levels of fibrinogen and factor VIII, though there was a trend for greater reduction to occur in the group with the lowest platelet count (<50,000/\( \mu l \)). The level of factor V was, however, significantly lower in those patients with the most severe thrombocytopenia (platelet count<50,000/\( \mu l \)) compared to those patients with moderate thrombocytopenia (platelet count 50,000–<150,000/\( \mu l \)) and the nontrombocytopenic patient (\( p < 0.05 \)). The level of factor XII was consistently reduced in all patients and there was a trend for marked reduction in patients with severe thrombocytopenia, although this was not statistically significant (Table 3).

Tests Reflecting Fibrin Formation and Lysis

FDP and PS were determined daily for 5–7 days from the onset of septicemia in 26 patients and to the

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yr)</th>
<th>Bacteria</th>
<th>Associated Disease</th>
<th>Antibiotics</th>
<th>Platelet Count (x 1000/( \mu l ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 4, 5, 12, 16</td>
<td>57–78</td>
<td>Escherichia coli</td>
<td>Cystitis, subdiaphragmatic abscess, cholecystitis</td>
<td>Ampicillin, gentamicin</td>
<td>2–94</td>
</tr>
<tr>
<td>6, 10, 24</td>
<td>60–75</td>
<td>Bacteroides fragilis</td>
<td>Peritonitis, large bowel perforation</td>
<td>Ampicillin, gentamicin</td>
<td>8–91</td>
</tr>
<tr>
<td>7, 3</td>
<td>58–76</td>
<td>Proteus mirabilis</td>
<td>Cystitis</td>
<td>Ampicillin, gentamicin</td>
<td>58–106</td>
</tr>
<tr>
<td>8, 17</td>
<td>41, 42</td>
<td>Klebsiella pneumoniae</td>
<td>Pneumonia, cystitis</td>
<td>Ampicillin, gentamicin</td>
<td>90–130</td>
</tr>
<tr>
<td>15</td>
<td>60</td>
<td>Enterobacter cloaceae</td>
<td>Ascending cholangitis</td>
<td>Ampicillin, gentamicin</td>
<td>65</td>
</tr>
<tr>
<td>18</td>
<td>65</td>
<td>Haemophilus influenzae</td>
<td>Acute cholecystitis</td>
<td>Ampicillin, gentamicin</td>
<td>135</td>
</tr>
<tr>
<td>29</td>
<td>39</td>
<td>Enterobacter aerogenes</td>
<td>Crohn’s disease</td>
<td>Gentamicin, cefamandole</td>
<td>145</td>
</tr>
<tr>
<td>Normal platelet count</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>78</td>
<td>Salmonella enteritidis</td>
<td>Osteomyelitis</td>
<td>Ampicillin, gentamicin</td>
<td>270</td>
</tr>
<tr>
<td>28</td>
<td>65</td>
<td>Pseudomonas aeruginosa</td>
<td>Cystitis</td>
<td>Gentamicin</td>
<td>180</td>
</tr>
</tbody>
</table>

Table 1. Data in Patients With Gram-Negative and Gram-Positive Septicemia

Disease Antibiotics

- Diabetes mellitus, acute bacterial endocarditis
- Penicillin, gentamicin (10–80)

- Pneumonia
- Penicillin, gentamicin (45)

- Infected pacemaker, diabetes mellitus, skin abscess, postsurgical infection
- Cefazolin (300–400)

- Pneumonia
- Penicillin, amoxicillin (285–280)

- Subacute bacterial endocarditis
- Penicillin (150)
Table 2. Platelet Survival in Septicemia

<table>
<thead>
<tr>
<th>Case No.</th>
<th>T½ Cr (h)</th>
<th>Platelet Count (x10³)</th>
<th>Bacterium</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>40</td>
<td>45.000</td>
<td>Strep. pneumoniae</td>
</tr>
<tr>
<td>5</td>
<td>57</td>
<td>94.000</td>
<td>E. coli</td>
</tr>
<tr>
<td>6</td>
<td>29</td>
<td>25.000</td>
<td>B. fragilis</td>
</tr>
<tr>
<td>21</td>
<td>82</td>
<td>150.000</td>
<td>Strep. viridans</td>
</tr>
<tr>
<td>26</td>
<td>78</td>
<td>330.000</td>
<td>Staph. aureus</td>
</tr>
</tbody>
</table>

*Normal range of T½ Cr is 80–120 hr.
†Normal range of platelet count is 150–450,000/µl.

Eight patients had a platelet count between 150,000 and 400,000/µl (cases 19–23, 26–28). One of these patients (case 23) had an elevated FDP level. The same patient had a positive PS test and an FPA level greater than 7.0 pmole/ml. The FPA value in the remaining 7 patients varied from 1.4 to 5.1 pmole/ml (mean 4.0, median 3.3).

Table 3. Levels of Fibrinogen and Factors V, VIII, and XII in Septicemic Patients

<table>
<thead>
<tr>
<th>Factor</th>
<th>Assay</th>
<th>Plasma Level</th>
<th>Platelet Count (≤ 50,000/µl)</th>
<th>Platelet Count (50,000–&lt; 150,000/µl)</th>
<th>Platelet Count (150,000–450,000/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor I</td>
<td>Mean</td>
<td>371</td>
<td>569</td>
<td>459</td>
<td></td>
</tr>
<tr>
<td>(mg/dl)</td>
<td>Range</td>
<td>174–630</td>
<td>315–900</td>
<td>244–663</td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>11</td>
<td></td>
<td>11</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Factor V</td>
<td>Mean</td>
<td>76*</td>
<td>114</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>(percent of normal)</td>
<td>Range</td>
<td>40–136</td>
<td>64–164</td>
<td>90–150</td>
<td></td>
</tr>
<tr>
<td>Factor VIII</td>
<td>Mean</td>
<td>167</td>
<td>240</td>
<td>238</td>
<td></td>
</tr>
<tr>
<td>(percent of normal)</td>
<td>Range</td>
<td>76–264</td>
<td>114–400</td>
<td>112–230</td>
<td></td>
</tr>
<tr>
<td>Factor XII</td>
<td>Mean</td>
<td>28.3</td>
<td>43.8</td>
<td>49.3</td>
<td></td>
</tr>
<tr>
<td>(percent of normal)</td>
<td>Range</td>
<td>13–40</td>
<td>16–77</td>
<td>23–70</td>
<td></td>
</tr>
</tbody>
</table>

*Significant difference, p < 0.05.
greatest reduction being in the patient with the lowest platelet count (T/2 29 hr, platelet count 25,000/mcl).

It has been suggested that the increased rate of platelet destruction in patients with sepsis may result from either overt or possible subclinical DIC.3,4,6 The results of this study show that while most patients with a platelet count of less than 50,000/mcl have laboratory evidence of intravascular coagulation, there are many patients with moderate thrombocytopenia in the range of 50,000–150,000/mcl where evidence of fibrin proteolysis measured by FDP, PS, and FGC tests was absent, though it is possible that other methods of performing these tests18 may have detected minor abnormalities. It is also possible that thrombocytopenia is a more sensitive indicator of in vivo thrombin action than are results of these protein coagulation assays. However, in vitro tests suggest that thrombin formed in solution reacts more rapidly with plasma fibrinogen than with platelet receptors.19 While the FPA was mildly elevated in many of these patients, it was similarly elevated in those patients with normal platelet counts. It seems most unlikely that increased thrombin action by itself accounts for the decreased platelet counts, since similar or higher FPA levels occur in patients with thromboembolism and other diseases who have platelet counts in the normal range.12,14 In a recent study of FPA levels and platelet counts in patients who received intrauterine infusion of hypertonic saline for termination of pregnancy, and in whom the decrease in platelet count may have more directly reflected increased thrombin action, a mean FPA level of 18 pmole/ml was associated with a mean drop in platelet count of 120,000/mcl.20 It is, therefore, likely that the thrombocytopenia (50,000–150,000/mcl) in this group of patients with septicemia is contributed to by mechanisms other than intravascular coagulation.

Endothelial cell damage occurs in endotoxin-treated animals21,22 and may be a contributing factor to thrombocytopenia in septicemic patients by promoting platelet adherence to subendothelium. The low levels of factor XII found in many of our patients may reflect activation and clearance of factor XII by exposed subendothelium23,24 or activation of factor XII by endotoxin.25 However, it is noteworthy that it occurred both in patients with gram-positive and gram-negative septicemia.

We have recently reported that many septicemic

---

**Fig. 1.** The relationship between tests reflecting fibrin formation and lysis and the patient's platelet count. Each of the results for FDP, FGC, and FPA represent the highest values obtained in each patient during serial testing. The results of PS are recorded positive if any of the serial tests were positive and are recorded as negative if none of the tests were positive. The platelet count shown is the lowest obtained for each patient.

**Table 4. Tests of Fibrin Formation and Lysis Related to Level of Platelet Count**

<table>
<thead>
<tr>
<th>Platelet Count</th>
<th>Abnormal FDP</th>
<th>Positive PS</th>
<th>Abnormal FGC</th>
<th>&gt;7.0 pmole/ml</th>
<th>FPA Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50,000</td>
<td>9/12*</td>
<td>10/12</td>
<td>7/8</td>
<td>2/2</td>
<td>18.6</td>
</tr>
<tr>
<td>50,000–&lt;150,000</td>
<td>1/11</td>
<td>3/11</td>
<td>3/11</td>
<td>1/7</td>
<td>5.5</td>
</tr>
<tr>
<td>150,000–400,000</td>
<td>1/8</td>
<td>1/8</td>
<td>0/7</td>
<td>1/8</td>
<td>4.0</td>
</tr>
</tbody>
</table>

* Number abnormal patients/number of patients tested.
patients with thrombocytopenia have an increased level of platelet-associated IgG. We have postulated that this may reflect the presence of immune complexes, comprised of bacterial products and their antibodies, which adhere to platelets and result in their premature destruction. Further clinical and animal studies are being undertaken to evaluate the prevalence and relative importance of thrombin action, platelet-associated IgG, and possible endothelial damage in the production of thrombocytopenia in septicemia.

ACKNOWLEDGMENT

We wish to thank Lee Dermody, Jean Russett, Marilyn Johnson, and members of the Department of Medicine, Hamilton General Hospital for their help.

REFERENCES

Thrombocytopenia in septicemia: the role of disseminated intravascular coagulation

PB Neame, JG Kelton, IR Walker, IO Stewart, HL Nossel and J Hirsh