

# Thrombocytopenia in Septicemia: The Role of Disseminated Intravascular Coagulation

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The mechanism of isolated thrombocytopenia in septicemia 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 septicemia and 8 of 14 patients with gram-positive septicemia had thrombocytopenia. Platelet survival studies demonstrated a decreased platelet survival. In 11 of 12 patients with severe thrombocytopenia (platelet count  $<50,000/\mu\text{l}$ ), 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  $<150,000/\mu\text{l}$ ) 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 septicemia.

COAGULATION abnormalities, ranging from a rapidly fulminant 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 septicemia.<sup>1,2</sup> Recent reports have emphasized that thrombocytopenia frequently occurs early in the course of septicemia without overt evidence of DIC in both adults<sup>3-5</sup> and children.<sup>6</sup> 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<sup>3,4,6</sup> and, in these circumstances, it has been suggested that subclinical or compensated DIC might be a contributing factor.<sup>3,4,6</sup>

We have performed a study to investigate the contribution of intravascular coagulation to the thrombocytopenia in septicemia. 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.

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## MATERIALS AND METHODS

### Patients

Thirty-one patients (ages 23–91 yr, mean 63 yr; M:F = 18:13) with septicemia associated with a variety of infections, including pneumonia, acute meningitis, 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 septicemia 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 <sup>51</sup>Cr-labeled homologous platelets.<sup>7</sup> The normal half-life (T<sub>1/2</sub> <sup>51</sup>Cr) using autologous platelets, in our laboratory, determined from 20 normal subjects, is 80–120 hr.

**Coagulation studies.** The following coagulation assays were performed: plasma fibrinogen,<sup>8</sup> factors V, VIII, and XII.<sup>9</sup>

**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  $\mu\text{l}/\text{ml}$ ). After centrifugation, 1000  $\mu$  bovine thrombin (Parke-Davis) and 0.1 M EACA plus 0.25 M CaCl<sub>2</sub> 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  $\mu\text{g}/\text{ml}$ .

(2) Protamine sulfate paracoagulation test (PS) for fibrin was performed by the method of Kidder et al.<sup>10</sup> 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.

(3) Fibrinogen gel chromatography (FGC) was performed on platelet-poor plasma by the method of Alkjaersig and Fletcher<sup>11</sup> 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.<sup>12</sup> in a heparin/trasyol mixture (4.5 ml blood and 0.5 ml heparin [500  $\mu$ ] and trasyol [500  $\mu$ ]), 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.<sup>13</sup>

### 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  $T_{1/2}$  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  $T_{1/2}$  was reduced in all three patients with thrombocytopenia and the patient with the lowest platelet count (25,000/ $\mu$ l) had the shortest platelet  $T_{1/2}$  (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 nonthrombocytopenic 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 1. Data in Patients With Gram-Negative and Gram-Positive Septicemia

Case No.	Age (yr)	Bacteria	Associated Disease	Antibiotics	Platelet Count ( $\times 1000/\mu$ l)
<b>Gram-negative septicemia</b>					
<b>Thrombocytopenic</b>					
1, 4, 5, 12, 16	57–78	<i>Escherichia coli</i>	Cystitis, subdiaphragmatic abscess, cholecystitis	Ampicillin, gentamicin	2–94
6, 10, 24	60–75	<i>Bacteroides fragilis</i>	Peritoneal dialysis, large bowel perforation	Ampicillin, gentamicin, Clindamycin	8–91
7, 13	58–68	<i>Proteus mirabilis</i>	Cystitis	Ampicillin, gentamicin, Cephalothin	58–106
8, 17	41, 42	<i>Klebsiella pneumoniae</i>	Pneumonia, cystitis	Ampicillin, gentamicin, Cloxacillin	90–130
15	60	<i>Enterobacter cloacae</i>	Ascending cholangitis	Ampicillin, gentamicin	65
18	65	<i>Haemophilus influenzae</i>	Acute cholecystitis	Ampicillin, gentamicin	135
29	39	<i>Enterobacter aerogenes</i>	Crohn's disease	Gentamicin, clindamycin	145
<b>Normal platelet count</b>					
27	78	<i>Salmonella enteritidis</i>	Osteomyelitis	Ampicillin, gentamicin	270
28	65	<i>Pseudomonas aeruginosa</i>	Cystitis	Gentamicin	180
<b>Gram-positive septicemia</b>					
<b>Thrombocytopenic</b>					
2, 9, 11, 14, 25, 30, 31	23–91	<i>Staphylococcus aureus</i>	meningitis, pneumonia, peritonitis, diabetes mellitus, acute bacterial endocarditis	Penicillin, cloxacillin, Ampicillin, gentamicin	10–80
3	73	<i>Streptococcus pneumoniae</i>	Pneumonia	Penicillin, gentamicin	45
<b>Normal platelet count</b>					
19, 22, 26	57–68	<i>Staphylococcus aureus</i>	Infected pacemaker, diabetes mellitus, skin abscess, postsurgical infection	Cloxacillin	300–400
20, 23	79–82	<i>Streptococcus pneumoniae</i>	Pneumonia	Penicillin, ampicillin	265–280
21	56	<i>Streptococcus viridans</i>	Subacute bacterial endocarditis	Penicillin	150

**Table 2. Platelet Survival in Septicemia**

Case No.	T½ <sup>51</sup> Cr*	Platelet Count†	Bacterium
3	40	45,000	<i>Strep. pneumoniae</i>
5	57	94,000	<i>E. coli</i>
6	29	25,000	<i>B. fragilis</i>
21	82	150,000	<i>Strep. viridans</i>
26	78	330,000	<i>Staph. aureus</i>

\*Normal range of T½ <sup>51</sup>Cr is 80–120 hr.

†Normal range of platelet count is 150–450,000/μl.

time of death in 5 patients. The FGC test was performed in 24 patients, at least once within 4 days after the diagnosis of septicemia. FPA levels were determined serially for 6 days or more in 16 patients and on one occasion in 1 patient who died. The relationship between the results of tests for FDP, PS, FGC, and FPA and the patient's platelet count is shown in Fig. 1.

In 12 patients, at least one platelet count was less than 50,000/μl. Laboratory evidence for DIC was present in 11 of these patients (Table 4). Nine of the 12 patients had elevated FDP (cases 2,4,6,10–12,14,30,31), 10 had a positive protamine sulfate test (cases 2,3,4,6,10–12,14,30,31), and 7 of 8 tested had increased levels of high molecular weight fibrin/fibrinogen complexes (cases 1,2,4,6,14,30,31). Two out of 2 tested had markedly elevated levels of FPA, greater than 7 pmole/ml (cases 14, 30), consistent with acute DIC.<sup>14</sup>

The lowest platelet count in 11 patients was between 50,000 and 150,000/μl. In contrast to the previous group, 7 of these patients had little evidence of intravascular fibrin formation or fibrinolysis. FDP levels were elevated in one patient (case 24), 3 had a positive protamine sulfate test and elevated fibrin/fibrinogen complexes (cases 5,7,24), and 1 of 7 tested had a markedly elevated level of FPA (case 13). The FPA value in the remaining 6 patients varied between 1.9 and 6.5 pmole/ml (mean 5.5, median 2.9).

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).

## DISCUSSION

A number of abnormalities of blood coagulation have been described in association with septicemia,<sup>1,2</sup> but the frequent occurrence of isolated thrombocytopenia only recently has been emphasized.<sup>3–6</sup> Thrombocytopenia occurs early in the course of septicemia and may alert the clinician to the possibility of septicemia since the platelet count is usually available before the pathogen is cultured from the blood;<sup>3</sup> an observation emphasized in 1966 in adults<sup>15</sup> and subsequently in children by Corrigan.<sup>6</sup>

The mechanism of thrombocytopenia in septicemia is uncertain. Its rapid onset suggests that there is increased platelet destruction, a mechanism that is supported by the results of platelet survival studies reported here and by others previously.<sup>15,16</sup> It is also likely that some impairment of platelet production occurs in patients with septicemia.<sup>17</sup>

It should be emphasized that for ethical reasons, the platelet survivals on the sick septicemic patients were performed using homologous platelets, while the normal control survivals were performed using autologous platelets. However, we do not feel that this alters the interpretation of our results. None of the patients had previously received blood products nor had they been pregnant. In addition, the platelet T½ in the two patients with normal platelet counts were at the lower limits of the normal range (78–82 hr). In contrast, the three patients with thrombocytopenia had reduced platelet survivals compared with our normal range, the

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

Factor Assay	Plasma Level	Platelet Count		
		(<50,000/μl)	(50,000–<150,000/μl)	(150,000–450,000/μl)
Factor I (mg/dl)	Mean	371	569	459
	Range	174–630	315–900	244–663
	Number	11	11	8
Factor V (percent of normal)	Mean	76*	114	110
	Range	40–136	64–164	90–150
	Number	12	11	7
Factor VIII (percent of normal)	Mean	167	240	238
	Range	76–264	114–400	112–230
	Number	12	11	7
Factor XII (percent of normal)	Mean	28.3	43.8	49.3
	Range	13–40	16–77	23–70
	Number	10	11	7

\*Significant difference,  $p < 0.05$ .

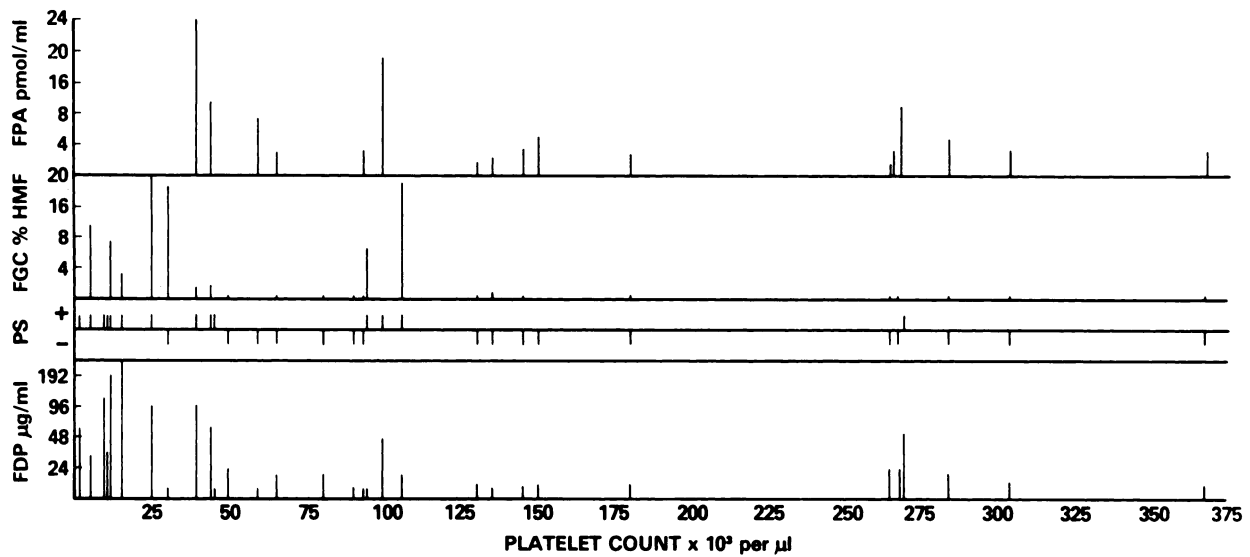


Fig. 1. The relationship between tests reflecting fibrin formation and lysis and the patient's platelet count. Each of the results for FDP, FGC, and FPA represent the highest values obtained in each patient during serial testing. The results of PS are recorded positive if any of the serial tests were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

greatest reduction being in the patient with the lowest platelet count ( $T_{1/2}$  29 hr, platelet count 25,000/ $\mu$ l).

It has been suggested that the increased rate of platelet destruction in patients with septicemia may result from either overt<sup>1</sup> or possible subclinical DIC.<sup>3,4,6</sup> The results of this study show that while most patients with a platelet count of less than 50,000/ $\mu$ l have laboratory evidence of intravascular coagulation, there are many patients with moderate thrombocytopenia in the range of 50,000–150,000/ $\mu$ l where evidence of fibrin proteolysis measured by FDP, PS, and FGC tests was absent, though it is possible that other methods of performing these tests<sup>18</sup> 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.<sup>19</sup> 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.<sup>12,14</sup> 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/ $\mu$ l.<sup>20</sup> It is, therefore, likely that the thrombocytopenia (50,000–150,000/ $\mu$ l) in this group of patients with septicemia is contributed to by mechanisms other than intravascular coagulation.

Endothelial cell damage occurs in endotoxin-treated animals<sup>21,22</sup> 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 subendothelium<sup>23,24</sup> or activation of factor XII by endotoxin.<sup>25</sup> However, it is noteworthy that it occurred both in patients with gram-positive and gram-negative septicemia.

We have recently reported that many septicemic

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

Platelet Count	Abnormal FDP	Positive PS	Abnormal FGC	FPA Level			
				> 7.0 pmole/ml	Mean	Median	Range
< 50,000	9/12*	10/12	7/8	2/2	18.6		10.7–26.5
50,000–<150,000	1/11	3/11	3/11	1/7	5.5	2.9	1.9–19.1
150,000– 400,000	1/8	1/8	0/7	1/8	4.0	3.3	1.4–9.3

\* Number abnormal patients/number of patients tested.

patients with thrombocytopenia have an increased level of platelet-associated IgG.<sup>26</sup> 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.

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