Fulminant Meningococcal Septicemia: Dissociation between Plasma Thrombopoietin Levels and Platelet Counts

Anna Bjerre, Reidun Øvstebo, Peter Kierulf, Sverre Halvorsen, and Petter Brandtzæg

From the Department of Pediatrics and the Research and Development Group, Department of Clinical Chemistry, Ullevål University Hospital, Oslo, Norway

Thrombopoietin (TPO), interleukin (IL)–6, and platelets were measured serially in 9 patients with fulminant meningococcal septicemia and consumption coagulopathy. The results were compared with those of patients with meningococcal meningitis and mild meningococcemia (n = 10) and with those of healthy control subjects (n = 19). TPO levels in control subjects were below the detection limit (<63 pg/mL). In patients with fulminant meningococcal septicemia, the median TPO level on admission was 193 pg/mL (range, 133–401 pg/mL), and the level peaked within 3–7 days (median, 488 pg/mL; range, 239–1334 pg/mL). Platelet counts remained low, despite the elevated TPO levels. In patients with meningitis or meningococcemia, the median TPO level on admission was 112 pg/mL (range, <63–695 pg/mL), and the TPO level was not detectable within 48 h. Platelet counts for these patients remained within normal limits. Maximum IL-6 levels in patients with septicemia were observed on admission (median, 5317 pg/mL; range, 188–651,000 pg/mL) and increased earlier than TPO levels. In patients with fulminant septicemia, TPO level increases significantly whereas the level of circulating platelets does not.

Fulminant meningococcal septicemia in humans represents an extreme form of endotoxin-induced sepsis and coagulopathy. The condition is diagnosed clinically by the appearance of hemorrhagic skin lesions and compromised circulation in a febrile patient. The size of the skin lesions can be used, to a certain extent, to predict the clinical severity and the ongoing coagulopathy [1]. The endotoxin levels observed in patients with meningococcal septicemia are significantly higher than those observed in patients with sepsis caused by other gram-negative bacteria, which may explain the extreme manifestations of this syndrome [2].

The massive endotoxemia induces cascades of proinflammatory mediators, including high levels of bioactive tumor necrosis factor–α, interleukin (IL)–1β, IL-6, and IL-8 [3, 4]. Of these, IL-6 is known to have thrombopoietic properties [5, 6].

The consumption coagulopathy is believed to be induced primarily by activation of the extrinsic pathway, as has been demonstrated in animal models that simulate severe gram-negative sepsis [7]. This hypothesis is supported by the observation that human monocytes collected from patients with fulminant meningococcal septicemia synthesize tissue factor (TF) and that high values of TF have been associated with disease severity and fatal outcome [8]. The intrinsic coagulation pathway is, however, also activated in these patients [9].

On hospital admission, patients with fulminant meningococcal septicemia usually reveal subnormal platelet counts, which decline during the first 24 h of hospitalization [10]. Thrombocytopenia is the rule during the first 7–10 days in surviving patients.

The process of platelet production is regulated by hematopoietic cytokines (IL-3, IL-6, IL-11, and thrombopoietin [TPO]) that act, either individually or in concert, to differentiate megakaryocytes into mature platelets [11]. TPO has recently been identified as a key regulator of platelet production [12–14], which stimulates both the proliferation and the maturation of megakaryocytes through binding to the receptor Mpl, which is encoded by the proto-oncogene. The regulatory mechanisms of platelet production are still not well understood. Because of the massive consumption of and abrupt decrease in circulating platelets during the early stage of fulminant meningococcal septicemia, we asked whether TPO is upregulated as a result of thrombocytopenia, and whether the level of IL-6 is related to the level of TPO. To examine these questions, we retrospectively measured TPO and IL-6 levels and platelet counts in serially collected plasma samples from patients with fulminant meningococcal septicemia. The results were compared with those obtained from patients with a bacteriologically proven meningococcal infection without coagulopathy and with those of healthy control subjects.
Table 1. Classification of patients and degree of coagulopathy on admission.

<table>
<thead>
<tr>
<th>Disease, patient</th>
<th>Platelets $\times 10^9$/L</th>
<th>Quicktime, s</th>
<th>APTT, s</th>
<th>Fibrinogen, g/L</th>
<th>FPA, $\mu$g/L</th>
<th>FDP, $\mu$g/L</th>
<th>LPS, ng/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fulminant septicemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>85</td>
<td>70</td>
<td>240</td>
<td>0.18</td>
<td>99</td>
<td>$&gt;80$</td>
<td>10,500</td>
</tr>
<tr>
<td>2</td>
<td>118</td>
<td>25</td>
<td>60</td>
<td>2.95</td>
<td>48</td>
<td>--</td>
<td>3800</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>--</td>
<td>60</td>
<td>1.3</td>
<td>--</td>
<td>40</td>
<td>3200</td>
</tr>
<tr>
<td>4</td>
<td>205</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>42</td>
<td>--</td>
</tr>
<tr>
<td>5</td>
<td>251</td>
<td>21</td>
<td>55</td>
<td>2.6</td>
<td>8</td>
<td>20</td>
<td>2800</td>
</tr>
<tr>
<td>6</td>
<td>162</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>17</td>
<td>--</td>
</tr>
<tr>
<td>7</td>
<td>74</td>
<td>21</td>
<td>54</td>
<td>2.15</td>
<td>--</td>
<td>80</td>
<td>1100</td>
</tr>
<tr>
<td>8</td>
<td>114</td>
<td>54</td>
<td>103</td>
<td>0.48</td>
<td>29</td>
<td>80</td>
<td>750</td>
</tr>
<tr>
<td>9</td>
<td>14</td>
<td>45</td>
<td>115</td>
<td>0.70</td>
<td>13</td>
<td>$&gt;80$</td>
<td>210</td>
</tr>
<tr>
<td>Median</td>
<td>114</td>
<td>35</td>
<td>60</td>
<td>1.3</td>
<td>29</td>
<td>80</td>
<td>2800</td>
</tr>
<tr>
<td>Meningitis/mild MC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>191</td>
<td>16</td>
<td>46</td>
<td>4.7</td>
<td>--</td>
<td>$&lt;10$</td>
<td>$&lt;25$</td>
</tr>
<tr>
<td>11</td>
<td>182</td>
<td>24</td>
<td>48</td>
<td>1.75</td>
<td>19</td>
<td>20</td>
<td>110</td>
</tr>
<tr>
<td>12</td>
<td>229</td>
<td>20</td>
<td>40</td>
<td>3.9</td>
<td>$&lt;10$</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>108</td>
<td>--</td>
<td>--</td>
<td>7.6</td>
<td>34</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>210</td>
<td>--</td>
<td>--</td>
<td>5.1</td>
<td>7</td>
<td>--</td>
<td>$&lt;25$</td>
</tr>
<tr>
<td>15</td>
<td>152</td>
<td>19</td>
<td>40</td>
<td>5.50</td>
<td>8</td>
<td>10</td>
<td>$&lt;25$</td>
</tr>
<tr>
<td>16</td>
<td>212</td>
<td>18</td>
<td>35</td>
<td>5.50</td>
<td>8</td>
<td>$&lt;10$</td>
<td>$&lt;25$</td>
</tr>
<tr>
<td>17</td>
<td>332</td>
<td>19</td>
<td>41</td>
<td>3.6</td>
<td>27</td>
<td>$&lt;10$</td>
<td>$&lt;25$</td>
</tr>
<tr>
<td>18</td>
<td>229</td>
<td>13</td>
<td>27</td>
<td>2.75</td>
<td>3</td>
<td>10</td>
<td>$&lt;25$</td>
</tr>
<tr>
<td>19</td>
<td>213</td>
<td>22</td>
<td>35</td>
<td>3.15</td>
<td>9</td>
<td>--</td>
<td>600</td>
</tr>
<tr>
<td>Median</td>
<td>211</td>
<td>19</td>
<td>40</td>
<td>4.3</td>
<td>8</td>
<td>10</td>
<td>$&lt;25$</td>
</tr>
</tbody>
</table>

NOTE. Values in parentheses are reference values. APTT, activated partial thromboplastin time; FDP, fibrin degradation products; FPA, fibrinopeptide A; LPS, lipopolysaccharide, endotoxin; MC, meningococcemia.

Patients and Methods

Nine patients with fulminant meningococcal septicemia were studied. All had positive blood cultures. One patient died within 48 h of admission to the hospital as a result of septic shock. Ten patients were included for comparison, 8 with meningococcal meningitis and 2 with mild meningococcemia. Six of these 10 patients had positive blood cultures. The 8 patients with meningococcal meningitis and 2 with mild meningococcemia were grouped together, since both clinical presentations are characterized by a low-grade intravascular inflammatory response, as measured by lipopolysaccharide levels and inflammatory mediators [2]. Data regarding the coagulopathy are given in table 1.

Control subjects. Nineteen healthy health care workers served as control subjects.

Clinical definitions. Meningococcal infection was considered to be present when Neisseria meningitidis was cultured from the blood and/or the CSF. Severe septic shock was defined as persistent hypoperfusion caused by bacterial infection, with an initial systolic blood pressure $<85$ mm Hg in adolescents and adults (aged $>12$ years) and $<70$ mm Hg in children (aged $<12$ years), that required fluid therapy and treatment with vasoactive drugs (dopamine or dopamine combined with epinephrine) for at least 24 h or until death [2]. Fulminant meningococcal septicemia was defined as a meningococcal infection that leads to rapidly evolving severe septic shock with minimal pleocytosis ($<10^6$ leukocytes/L CSF; patients 1–9, table 1). Meningococcal meningitis was defined as a meningococcal infection with marked pleocytosis ($>10^6$ leukocytes/L CSF) and absence of septic shock (patients 10–17, table 1). Mild meningococcemia was defined as a meningococcal infection, that is, N. meningitidis in blood culture(s), without persistent septic shock or meningitis (patients 18 and 19, table 1).

TPO. Blood collected in EDTA vacuum tubes (Vacutainer; Becton Dickinson, Meylan Cedex, France) was centrifuged (1400 $\times$ g for 10 min), and the plasma was stored in cryotubes (Nunc, Roskilde, Denmark) at $-70^\circ$C. TPO in plasma was analyzed by use of an ELISA method (Quantikine human TPO immunoassay, R&D Systems, Abingdon, UK). The detection limit was 63 pg/mL.

Platelets were counted automatically in EDTA blood (Vacutainer) by use of Ortho ELT-800/WS (Ortho Diagnostic Systems, Westwood, MA). Reference values were $150-400 \times 10^9$/L.

Figure 1. Plasma thrombopoietin (TPO) levels during the first week of fulminant meningococcal septicemia in 8 patients (1 patient died within 48 h). Horizontal dotted line indicates the detection limit. Symbols indicate levels for individual patients.
IL-6. Blood samples were collected and processed as described elsewhere and were analyzed by an ELISA method (Quantikine). The detection limit was 0.70 pg/mL.

Statistical analysis. The differences between groups were calculated with Wilcoxon rank sum test for unpaired data. \( P < .05 \) was considered statistically significant.

Results

TPO levels and platelet counts were analyzed in sequentially collected samples during the first 7–10 days of disease. In samples collected within 24 h of admission, the median TPO levels were 193 pg/mL (range, 133–401 pg/mL) for patients with fulminant septicemia and 112 pg/mL (<63–695 pg/mL) for patients with meningitis or mild meningococcemia, respectively (\( P = .3 \)). None of the 19 healthy control subjects had detectable (<63 pg/mL) TPO in plasma; therefore, plasma TPO levels in patients were significantly higher than those in healthy control subjects (\( P < .001 \)).

The TPO levels increased rapidly in patients with fulminant septicemia, reaching peak values on days 3–7. TPO levels then gradually decreased, reaching normal values during the second week (figure 1). The median peak TPO level in patients with fulminant septicemia was 488 pg/mL (range, 239–1334 pg/mL; figure 2). Two patients with meningitis/mild meningococcemia had initially markedly elevated TPO levels. The levels declined within 48 h (data not shown). The peak value was 144 pg/mL (range, 63–695 pg/mL; figure 2). The peak TPO values differed significantly between the 2 patient groups (\( P = .008 \)).

Median platelet counts on admission were \( 114 \times 10^9/\text{L} \) (range, 14–251 \( \times 10^9/\text{L} \)) for patients with septicemia and \( 211 \times 10^9/\text{L} \) (range, 108–332 \( \times 10^9/\text{L} \)) for patients with meningitis/mild meningococcemia (figure 3). The difference was statistically significant (\( P = .01 \)). Median platelet counts in healthy control subjects were \( 229 \times 10^9/\text{L} \) (range, 152–329 \( \times 10^9/\text{L} \); figure 3). Despite high levels of TPO, patients with septicemia did not demonstrate a significant increase in platelet counts, as indicated for 3 different patients in figure 4.

Median peak IL-6 values were 5317 pg/mL (range, 188–651,000 pg/mL) for patients with fulminant septicemia and 67 pg/mL (range, 18–382 pg/mL) for patients with meningitis and mild disease. The difference was statistically significant (\( P = .0001 \)). IL-6 peak values preceded those of TPO and declined before TPO peak values (figure 5).

Discussion

TPO is upregulated from an early stage in patients with fulminant meningococcal septicemia and levels continue to increase during the first 3–7 days. Six of 9 patients had a subnormal platelet count on admission. Platelet counts continued to decrease after treatment was initiated and remained low (<50 \( \times 10^9/\text{L} \)) for 7–10 days in 7 of 9 patients, despite grossly elevated TPO levels.

TPO is accepted to be the main regulator of platelet production and maturation [12–14]. An inverse correlation between platelet count and plasma TPO levels has been observed, but TPO levels correlate better with the platelet mass [15], and probably with megakaryocyte mass [16], than with the platelet count per se. Results of animal studies suggest that TPO is constantly produced in the liver and kidney [17], but that circulating levels of TPO are not modulated by changes in TPO gene expression in the liver or kidney [18]. Plasma TPO seems to be removed by binding to the c-Mpl receptor on platelets and megakaryocytes [17].

Administration of recombinant human TPO to cancer patients increases their circulating platelet counts in a dose-dependent manner [19]. In another study of cancer patients, the peak TPO levels were reached at platelet nadir, and TPO returned to normal values when the platelet count normalized.
In our patients with fulminant septicemia, we observed an inverse relationship between circulating platelet counts and levels of TPO. Contrary to the findings in the cancer patients, however, TPO declined in patients with fulminant septicemia long before an increase in circulating platelets was observed.

Patients with fulminant meningococcemia have extensive formation of fibrin thrombi in certain tissues (i.e., adrenal glands, skin, kidneys, and lungs [21]). A significant number of platelets is probably trapped in the vascular bed by fibrin thrombi and altered endothelial cells. Thus, platelet production in the bone marrow may increase but not be detected in the peripheral blood because of increased platelet consumption. It remains unknown to what extent fibrin-trapped platelets may release TPO on disintegration. On the other hand, elevated platelet turnover might remove TPO by ligation to c-Mpl receptors on platelets.

TPO has many structural similarities with erythropoietin (EPO), among these also in the receptor-binding domain [22]. The regulatory mechanisms of TPO are, however, quite different from those of EPO. Animal studies suggest that TPO is constitutively produced, whereas hypoxia stimulates EPO gene expression resulting in elevated EPO production [23]. Patients with sepsis and septic shock have been shown to have an elevated level of EPO in plasma, which is preceded by an elevated IL-6 level [24, 25]. Two previous studies suggest that IL-6 may augment EPO production in critically ill children [24, 25]. On admission, our patients had a massive upregulation of IL-6 that preceded the increase of TPO levels. Waage et al. [26] have shown that IL-6 in the same group of patients was bioactive, by employing a IL-6 dependent mouse hybridoma cell assay [26]. Thus the increasing levels of TPO in patients with fulminant meningococcal septicemia may be a consequence of the proinflammatory cytokine response, a response that includes IL-6 and rapidly declines after initiation of treatment.

In a recently published study of children with Kawasaki disease [27], TPO levels peaked on day 6 (± 2 days) after admission. These patients had normal platelet counts during the first week of the disease but revealed a marked thrombocytosis during the second week. Our patients with fulminant meningococcal septicemia had TPO levels that increased in a pattern similar to those of the patients with Kawasaki disease. However, we did not observe any increase in the platelet count after the TPO levels increased.

The intravascular inflammatory response observed in patients with fulminant meningococcal septicemia is extremely complex, comprising a variety of proinflammatory, as well as anti-inflammatory, principles [4]. In a recent study that evaluated the net inflammatory capacity of shock plasma from patients with meningococcal septicemia using human monocytes as a target cell, the anti-inflammatory principles primarily represented by IL-10 appeared to prevail over the proinflammatory principles [28]. The net effect of such plasma on human megakaryocytes remains to be elucidated. One cannot rule out the possibility that IL-10 may have a temporary bone marrow suppressive effect on megakaryocytes, counteracting the stimulatory effect of TPO.

Finally, our TPO results have been based on demonstrating elevated TPO antigen levels. To what extent this antigen has biological activity in septicemic patients remains unknown. A TPO responsive cell line system could expand further on this.

References


Figure 4. Platelet and thrombopoietin (TPO) levels in 3 patients with fulminant meningococcal septicemia. ●, platelets; ▲, TPO.

Figure 5. Thrombopoietin (TPO) and interleukin (IL)-6 levels in 4 patients with fulminant meningococcal septicemia. □, IL-6; ▲, TPO.


