Activation of the Coagulation Cascade in Patients with Leptospirosis

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**Background.** Disseminated intravascular coagulation (DIC) is common among patients with sepsis. Leptospirosis is an important cause of sepsis in tropical areas, and pulmonary hemorrhage associated with thrombocytopenia is the major cause of death, but the coagulopathy in severe leptospirosis has not been further characterized. The aim of this study was to evaluate coagulation factors and the presence of DIC in patients with leptospirosis in northeast Thailand.

**Methods.** We measured plasma concentrations of fibrinogen, D-dimer, thrombin-antithrombin III complexes, and prothrombin fragment 1,2 and evaluated the DIC score in 79 patients with culture-confirmed and/or serologically confirmed leptospirosis and in 33 healthy Thai control subjects.

**Results.** The median concentrations of fibrinogen, D-dimer, thrombin-antithrombin III complexes, and prothrombin fragment 1,2 were significantly elevated in a cohort of 79 patients with leptospirosis, compared with healthy control subjects (P < .001 for all tests). Patients with leptospirosis had significantly longer prothrombin times, longer activated partial thromboplastin times, and lower platelet counts. Thrombocytopenia was present in 38% of case patients and occurred more frequently among patients with culture-negative leptospirosis; in multivariate analysis, it was the only hemostasis factor independently associated with clinical bleeding. Patients who were culture-negative for *Leptospira* species had higher Acute Physiology and Chronic Health Evaluation II and Sepsis-Related Organ Failure Assessment scores and more bleeding complications. Nearly one-half of patients with leptospirosis had overt DIC as defined by an International Society on Thrombosis and Hemostasis DIC score.

**Conclusions.** Activation of the coagulation system is an important feature of leptospirosis. Thrombocytopenia is an indicator of severe disease and risk of bleeding.

Leptospirosis is a worldwide zoonosis caused by pathogenic members of the genus *Leptospira*. A variety of wild and domestic animals excrete the organism in their urine. Human infection occurs through direct contact with infected animals or through exposure to fresh water or soil contaminated by infected animal urine. Leptospirosis is recognized as an important emerging infection in many countries, including Thailand, where a recent major outbreak of the disease has been reported in the northern and northeastern regions [1, 2].

The clinical manifestations of leptospirosis range from a mild, often self-limiting flu-like illness to an acute, life-threatening multisystem disorder. The complications of severe disease include renal failure, abnormal liver function test results, jaundice, hypotension, and pulmonary hemorrhage. The common causes of death are pulmonary hemorrhage, protracted shock, and renal failure [3]. The pathophysiological mechanisms responsible for bleeding in leptospirosis are poorly understood. Thrombocytopenia has been reported to occur in 50%–80% of patients and is usually associated with severe disease with renal failure [4], but it was not associated with higher mortality in one retrospective study [5]. A prospective study showed that low platelet count was not a risk factor associated with...
mortality in patients with leptospirosis and acute pulmonary injury [6]. Findings in animal studies have been conflicting, in that some studies have shown evidence of disseminated intravascular coagulation (DIC) [7–9], whereas others have not [10, 11].

The aim of this study was to determine the frequency and clinical associations of DIC in patients with leptospirosis. Fibrinogen, cross-linked fibrin degradation products (D-dimer), thrombin-antithrombin III complexes (TAT), and prothrombin fragment 1,2 (F1+2) were measured in patients with confirmed disease during an outbreak of leptospirosis in northeast Thailand.

PATIENTS AND METHODS

Patients and study design. A prospective study was performed from October 2000 through September 2001 to identify patients with leptospirosis. The study protocol was approved by the Ethical Committee of the Ministry of Public Health, Royal Government of Thailand. Consecutive patients (≥14 years of age) who were admitted with an acute febrile illness to Udon Thani Regional Hospital in Udon Thani, northeast Thailand, were recruited after giving informed consent. History and physical examination findings were recorded on a clinical record form. Blood samples were taken from all patients for baseline laboratory testing, including a complete blood count, renal and liver function tests, and conventional blood cultures. A 10-mL blood sample was also collected into a sterile tube containing 250 U of heparin sodium (Heparin Leo; Leo Pharma) for Leptospira culture on the day of hospital admission. CSF samples from patients with neck stiffness was also cultured for aerobic bacteria and Leptospira species. Serum samples were obtained at hospital admission, days 3 and 7, and weekly until discharge from the hospital. If patients were discharged <2 weeks after hospitalization, they were asked to return for a follow-up visit 1 week after discharge from the hospital.

Culture for Leptospira was performed using Ellinghausen-McCullough-Johnson-Harris media supplemented with 3% rabbit serum and 0.1% agarose, as described elsewhere [12]. Samples with positive culture results were sent to the World Health Organization/Food and Agriculture Organization of the United Nations/World Organisation for Animal Health Collaborating Center for Reference and Research on Leptospirosis (Brisbane, Australia) for serovar identification using the cross-agglutinin absorption test [13]. Serological testing for leptospirosis was performed using the microscopic agglutination test at the National Institute of Health and National Institute of Animal Health, Ministry of Public Health, Thailand (Bangkok, Thailand). A diagnosis of leptospirosis was made if CSF or blood cultures became positive for Leptospira species and/or when there was a ≥4-fold increase in titers between acute-phase and convalescent-phase serum samples or a single titer of ≥1:400.

Blood samples were obtained from all patients for coagulation testing at admission to the hospital, to compare with blood samples obtained from 33 healthy Thai volunteers who were known to have no illness that could affect hemostasis and were not currently receiving any medication. Blood samples were collected into 3.2% sodium citrate tubes (BD Vacutainer Blood Collection Tube; Becton Dickinson). Plasma was separated into 2 tubes; 1 was used for immediate coagulation testing, and 1 was stored immediately at −70°C until the assays were performed.

A primary analysis was performed in which results for control subjects and patients with leptospirosis were compared. Subgroup analysis was performed to compare results from patients with cultures positive for Leptospira species with those from culture-negative patients and to compare results from patients with severe disease with those from patients with non-severe disease. Patients were defined as having severe leptospirosis if ≥1 of the following were present: renal involvement (oliguria or a creatinine level ≥2.5 mg/dL), jaundice (total bilirubin level ≥2.5 mg/dL), clinical bleeding abnormalities, hypotension (systolic blood pressure <90 mmHg or diastolic blood pressure <60 mmHg), or the presence of respiratory distress or respiratory failure. Patients were defined as having thrombocytopenia if they had a platelet count of ≤100 × 10⁹ platelets/mL. A DIC score for each patient was also calculated using the platelet count and the D-dimer, prothrombin time (PT), and fibrinogen levels according to the algorithm for the diagnosis of overt DIC recommended by the DIC scientific subcommittee of the International Society for Thrombosis and Hemostasis [14]. This DIC score was calculated by assigning 1 point for each of the following: (1) a platelet count of <100 but ≥50 × 10⁹ platelets/mL, (2) a prolonged PT of >3 s but <6 s, and (3) a fibrinogen level <1.0 g/L. A platelet count ≤50 × 10⁹ platelets/mL, a prolonged PT of ≥6 s, and an elevated D-dimer ≥2 times but <5 times the upper limit of normal were each assigned 2 points, and a D-dimer of ≥5 times the upper limit of normal was assigned 3 points. A score ≥5 was compatible with overt DIC, whereas a score of <5 may be indicative (but is not affirmative) of nonovert DIC. The algorithm for nonovert DIC was not applied, because protein C and antithrombin levels were not measured. No follow-up coagulation tests were performed in this study. Clinical severity was assessed using the APACHE II score [15] and Sepsis-Related Organ Failure Assessment (SOFA) score [16].

Assays. PT and activated partial thromboplastin time were performed at Udon Thani Regional Hospital (Udon Thani, Thailand) on the day of hospital admission using the Thromborel S and Dade Actin FS Activated PTT reagents (Dade Behring), respectively. Stored citrated plasma was transferred on
Table 1. Baseline information for 79 patients with leptospirosis, comparing culture-positive and culture-negative groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>All (n = 79)</th>
<th>Culture positive for Leptospira species (n = 48)</th>
<th>Culture negative for Leptospira species (n = 31)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>38.5 (15–72)</td>
<td>35 (15–72)</td>
<td>41 (20–67)</td>
<td>NS</td>
</tr>
<tr>
<td>Male sex, no. (%) of patients</td>
<td>58 (73.4)</td>
<td>36 (75)</td>
<td>22 (71)</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of symptoms, days</td>
<td>4 (2–8)</td>
<td>3 (2–8)</td>
<td>5 (2–8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>77 (37–113)</td>
<td>80 (37–113)</td>
<td>73 (46–112)</td>
<td>NS</td>
</tr>
<tr>
<td>Blood urea nitrogen level, mmol/L</td>
<td>7.9 (2.5–36.4)</td>
<td>6.4 (2.5–32.1)</td>
<td>13.9 (2.9–36.4)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Serum creatinine level, µmol/L</td>
<td>133 (62–955)</td>
<td>124 (71–636)</td>
<td>283 (62–955)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Serum bicarbonate level, µmol/L</td>
<td>23 (11–31)</td>
<td>25 (15–31)</td>
<td>21 (11–29)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Serum potassium level, µmol/L</td>
<td>3.6 (1.8–7)</td>
<td>3.6 (2.5–4.9)</td>
<td>3.6 (1.8–7.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Total serum bilirubin level, µmol/L</td>
<td>24 (5–397)</td>
<td>19 (5–164)</td>
<td>65 (7–397)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Direct serum bilirubin level, µmol/L</td>
<td>14 (3.4–243)</td>
<td>10 (3.4–111)</td>
<td>39 (3.4–243)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Aspartate aminotransferase level, U/L</td>
<td>61 (13–326)</td>
<td>52 (13–213)</td>
<td>70 (20–326)</td>
<td>.06</td>
</tr>
<tr>
<td>Alanine aminotransferase level, U/L</td>
<td>42 (9–201)</td>
<td>49 (9–201)</td>
<td>41 (19–142)</td>
<td>NS</td>
</tr>
<tr>
<td>Alkaline phosphatase level, U/L</td>
<td>108 (43–446)</td>
<td>96 (52–446)</td>
<td>120 (43–391)</td>
<td>NS</td>
</tr>
<tr>
<td>Albumin level, g/L</td>
<td>36 (20–48)</td>
<td>39 (28–48)</td>
<td>30 (20–40)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Globulin level, g/L</td>
<td>30 (19–54)</td>
<td>31 (23–44)</td>
<td>27 (19–54)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Hemoglobin level, mmol/L</td>
<td>129 (70–168)</td>
<td>134 (99–168)</td>
<td>119 (70–145)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>WBC count, (\times 10^9) cells/L</td>
<td>11.0 (5.2–33.5)</td>
<td>11.4 (5.8–28.3)</td>
<td>10.7 (5.2–33.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Neutrophil percentage</td>
<td>86 (45–95)</td>
<td>86 (70–95)</td>
<td>86 (45–94)</td>
<td>NS</td>
</tr>
</tbody>
</table>

**NOTE.** Data are given as median value (range), unless otherwise indicated. NS, not significant.

RESULTS

**Patients.** A total of 79 consecutive patients with confirmed leptospirosis were enrolled in the study. Of these, 48 (61%) had cases that were diagnosed by isolation of *Leptospira* species in either blood or CSF samples (45 from blood samples, 2 from blood and CSF samples, and 1 from CSF samples only), and 31 patients (39%) were culture negative and had cases that were diagnosed on the basis of microscopic agglutination test results. The serovar determinations for cultured *Leptospira* species were as follows: *Leptospira interrogans* serovar Autumnalis (32 isolates), *L. interrogans* serovar Pyrogenes (3), *L. interrogans* serovar Javanica (1), *L. interrogans* serovar Medanensis (2), and unidentified serovar (10). A summary of baseline clinical features and baseline laboratory results are shown in table 1. There were 2 deaths (2.5%) due to pulmonary hemorrhage in the group of patients who were culture positive for *Leptospira* species. Patients who were not leptospiremic at admission to the hospital had a longer median duration of symptoms prior to admission and had more-severe manifestations in terms of blood chemistry reflecting renal and liver impairment, higher APACHE II score, and a higher proportion of clinical bleeding manifestations (tables 1 and 2).
Platelets and coagulation tests. Patients with leptospirosis had a significantly lower median (interquartile range) platelet count than did the control group (142.5 ± 10^9 platelets/mL [30–211 ± 10^9 platelets/mL] vs. 289 ± 10^9 platelets/mL [228–328 ± 10^9 platelets/mL]; P < .001). They also had significantly longer median PTs and activated partial thromboplastin times and had higher median levels of D-dimer, F1+2, and TAT (P < .001) (figure 1). These differences were preserved in subgroup analyses, in which culture-negative and culture-positive groups were compared separately with the control group.

Coagulation, DIC score, and clinical severity. Forty-five patients (57%) presented with severe complications of leptospirosis (oliguria or renal failure in 24 patients, jaundice in 26, abnormal bleeding in 18, hypotension in 23, and respiratory distress or failure in 6). These patients had a significantly lower median (interquartile range) platelet count than did patients with less severe complications (66.5 ± 10^9 platelets/mL [33.5–166 ± 10^9 platelets/mL] vs. 192 ± 10^9 platelets/mL [144–240 ± 10^9 platelets/mL]; P < .001), but the other coagulation test results and factors were not different between the 2 groups (data not shown). The platelet count was correlated negatively with the APACHE II and SOFA scores (for APACHE II score, |ρ| = 0.53 and P < .001; for SOFA score, |ρ| = 0.75 and P < .001), whereas the other coagulation parameters had no significant correlations with APACHE II and SOFA scores. The median (interquartile range) overt DIC score was significantly higher for patients with severe leptospirosis than it was for patients with less severe leptospirosis (6 [5–6] vs. 4 [3–5]; P = .003). Thirty-six patients (46%) had an overt DIC score ≥ 5, which is compatible with DIC. The proportion of patients with an overt DIC score ≥ 5 was higher among patients who had severe disease than it was among patients with less severe disease (27 [60%] of 45 vs. 9 [26%] of 34; P = .003). In a logistic regression analysis considering platelet count, D-dimer, F1+2, and TAT, platelet count was the only factor independently associated with clinical bleeding (P = .003).

Coagulation, DIC score, and clinical bleeding. Eighteen patients had clinically significant bleeding. The median (interquartile range) APACHE II, SOFA, and overt DIC scores were higher in patients with abnormal bleeding than in those who did not have abnormal bleeding, as follows: for APACHE II score, 10 (7–15) versus 5 (3–9) (P = .004); for SOFA score, 14 (10–16) versus 2 (1–6) (P < .001); and for overt DIC score, 5 (3–6) versus 4 (3–5) (P = .001). Patients with clinically significant bleeding had significantly lower platelet counts, compared with the nonbleeding group (median platelet count, 43.5 vs. 162 ± 10^9 platelets/mL; P < .001). The clinical bleeding group also had a higher proportion of patients with thrombocytopenia (14 [78%] vs. 16 [27%]; P < .001). The other coagulation test results and markers were not different between the patients who had bleeding and those who did not (P ≥ .15). There was no difference in mortality between the 2 groups.
Figure 1. Platelet count and coagulation factors in patients with leptospirosis (empty triangles) who were culture negative (black triangles) or culture positive (black diamonds) for Leptospira species and in healthy Thai control subjects (empty squares).
DIC score/H11091

rubin ( ), and aspartate aminotransferase ( ) lev-
confirmed. ( ), microangiopathic hemolytic anemia could not be
less than did those who had normal platelet counts (data not

(1 [5.6%] in the bleeding group and 1 [1.6%] in the nonbleed-
ing group; ). The proportion of patients with an overt DIC score \( \geq 5 \) was higher among patients who had clinical bleeding than it was among those with no clinically significant bleeding (14 [78%] of 18 vs. 22 [36%] of 61; \( P = .002 \)).

DISCUSSION

Abnormal bleeding is not uncommon in leptospirosis, and it is

often present in fatal cases. The most common site is the lung, and other complications, such as intracerebral hemorrhage, occur only rarely [17]. Immunofluorescence studies performed on infected lung tissue specimens in the experimental leptospirosis guinea pig model have shown the presence of IgM, IgG, IgA, and C3 along the alveolar basement membrane, suggesting a possible role for an autoimmune process in fatal pulmonary hemorrhage associated with leptospirosis [10].

Thrombocytopenia is frequently found in leptospirosis but, to our knowledge, has not previously been correlated directly with a higher incidence of clinical bleeding [18, 19]. In this study, patients with clinical bleeding had significantly lower platelet counts than did other patients. The mechanism of thrombocytopenia in leptospirosis is not well defined. Some studies have suggested defects in production caused by a direct toxic effect of the organism on the bone marrow [20, 21]. Several studies have shown nonimmune platelet destruction to be an effect of DIC, immune-mediated causes that respond to treatment with methylprednisolone and hydrocortisone [22], and increased consumption of platelets secondary to activation of vascular endothelium [19]. Recent studies involving a guinea pig model also showed evidence of platelet activation as reflected by increased plasma levels of 11-dehydrogenate thromboxane B2 without significant increases of D-dimer, TAT complexes, and fibrinogen degradation products and lack of platelet and fibrin thrombi formation, suggesting that, in this animal model, the mechanism of bleeding was not DIC [11].

Other thrombocytopenic syndromes, such as hemolytic uremic syndrome and thrombotic thrombocytopenic purpura, can cause thrombocytopenia and have been reported rarely in leptospirosis [23, 24]. Thrombocytopenia and renal impairment, which are characteristic features of these syndromes, were found commonly in this study (in 38% and 26% of patients, respectively), but neurological symptoms, such as those seen in thrombotic thrombocytopenic purpura, were found in <10% of patients. Unfortunately, peripheral blood films were not performed in this study, so the presence of schistocytes could not be assessed. Although the patients with thrombocytopenia had lower hemoglobin concentrations ( \( P = .002 \) ) and higher bilirubin ( \( P < .001 \) ) and aspartate aminotransferase ( \( P = .004 \) ) levels than did those who had normal platelet counts (data not shown), microangiopathic hemolytic anemia could not be confirmed.

Fibrinogen is an acute-phase reactant, and plasma levels may be increased or remain normal during infection, despite ongoing consumption in the process of DIC. Hypofibrinogenemia occurs infrequently in severe cases of DIC, with a reported sensitivity of only 28% of a low fibrinogen level as a predictor of DIC [25, 26].

A recent review of coagulation disorders and the pathogenesis of leptospirosis concluded that the bleeding tendency results from an imbalance in the hemostatic equilibrium, caused by as yet unknown mechanisms. This hemostatic imbalance may lead to DIC, but this needs to be proven in a prospective study [27]. DIC is a complex, systemic thrombohemorrhagic syndrome, which is difficult to diagnose and for which there is no single diagnostic test. However, using the diagnostic algorithm introduced by the subcommittee on DIC of the International Society for Thrombosis and Hemostasis in 2001 (with a reported 91% sensitivity and 97% specificity) [14], we found that nearly one-half of the patients with leptospirosis had a DIC score \( \geq 5 \), indicative of overt DIC. We were unable to evaluate this DIC score as a predictor of fatal outcome because of the very low mortality (2.5%) among the study patients.

Patients with blood cultures positive for Leptospira species presented earlier in the course of their illness than did those with negative culture results; therefore, antibiotic treatment was also started earlier. This may explain why, as a group, they had less severe disease manifestations in terms of lower APACHE II and SOFA scores, higher platelet counts, fewer cases of thrombocytopenia, fewer clinical bleeding complications, and lower DIC scores. These clinically important differences between patients who are culture positive for leptospirosis and those who are culture negative have not been described previously.

In summary, activation of the coagulation cascade is common during leptospirosis, and this is often associated with DIC, which occurred in nearly one-half of study patients. However, thrombocytopenia was the only hemostasis-related factor independently associated with clinical bleeding, and it should be considered to be a predictor of disease severity in patients with leptospirosis.

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