Levels of procalcitonin (ProCT), a prohormone of calcitonin, have been found to be elevated in individuals with bacterial infections. In sepsis, ProCT levels correlate well with the severity of disease and outcome. Studies in healthy volunteers and in sepsis have shown close relationships with endotoxin, tumor necrosis factor (TNF-α), and interleukin-6 (IL-6). These cytokines, as well as macrophage inflammatory protein (MIP-1α), soluble TNF receptor, and IL-8, are related to clinical and biological markers of severity in malaria. The effects of TNF-α are thought to be crucial in the development of cerebral malaria. The induction of fever, prostaglandin E3 synthesis, activation of endothelium, neutrophils, macrophages, and lymphocytes are all modulated by TNF. Since TNF and endotoxin are thought to be among the main inductors of ProCT synthesis, we examined levels of ProCT in patients with complicated Plasmodium falciparum malaria and the effect of artesunate and mefloquine treatment on initial levels.

MATERIALS AND METHODS

The study was conducted at the Hospital for Tropical Diseases in Bangkok, Thailand. Transmission of malaria in this region is of the focal forest fringe type, affecting mainly adult migrant workers. Patients meeting the following criteria were included into the study: age between 15 and 65 years, infection with P. falciparum only, no use of chemotherapeutic drugs during the preceding 14 days, and symptoms of severe and complicated malaria as defined by the World Health Organization criteria. The study was approved by the Ethical Board of Mahidol University, and all patients gave informed consent before enrollment in the study.

All patients were treated with intravenous artesunate or artemisinin derivatives followed by mefloquine as part of therapeutic drugs during the preceding 14 days, and symptoms of severe and complicated malaria as defined by the World Health Organization criteria. The study was approved by the Ethical Board of Mahidol University, and all patients gave informed consent before enrollment in the study.

Blood samples for routine laboratory parameters, for thick and thin smears, and for the determination of ProCT levels were taken on admission and on days 7, 14, 21, and 28. Routine examinations included red blood cell count, hematocrit, white blood cell count, platelet count, serum electrolytes, bilirubin, serum creatinine, and liver enzymes. Blood smears (thick and thin films) were obtained from fingersticks, stained with Giemsa, and parasite counts were performed. Parasite density was determined by counting the number of parasites per 1,000 red blood cells in a thick film or the number of parasites per 200 white cells in a thick film. The number of parasites per microliter of blood was calculated from these figures. Blood films were declared negative if no parasites were seen in 200 oil-immersion fields on a thick film. Blood smears were prepared and examined every 6 hr until they were negative for two consecutive examinations; thereafter, smears were prepared and examined daily.

Blood samples for determination of procalcitonin were taken on admission, and on days 7, 14, 21, and 28. Serum was stored at −20°C until analysis. Levels of ProCT were determined using an immunoluminometric assay (LUMITEST® PCT; Brahms Diagnostica GmbH, Berlin, Germany). This coated tube assay uses two antigen-specific monoclonal antibodies reacting with the calcitonin and the kacticulin part of ProCT, respectively. Both antibodies react with ProCT, forming sandwich complexes. After the samples were washed five times with a washing solution provided by the manufacturer, the remaining luminescence was measured using a standard luminometer. Functional assay sensitivity was 0.3 ng/ml. Values < 0.5 ng/ml were considered normal.

Nitrite/nitrate was measured using high-performance liquid chromatography (HPLC) (Shimadzu, Tokyo, Japan). For analysis, samples were deproteinized with trifluoroacetic acid and centrifuged. Five hundred microliters of supernatant was mixed with 100 μl of 5% NaOH and 400 μl of double-distilled water. This solution was loaded onto a solid-phase extraction C-18 column and a solid-phase ion exchanger column and finally eluted with 200 μl of a 0.5 M solution of the mobile phase. Twenty microliters was injected into the HPLC column through the auto injector. The minimal detectable concentration was 0.1 nmol/L.
TABLE 1
Routine laboratory parameters on admission

<table>
<thead>
<tr>
<th>Parameter*</th>
<th>Unit</th>
<th>Normal range</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets</td>
<td>× 10⁹/L</td>
<td>150–350</td>
<td>68.89</td>
<td>51.09</td>
</tr>
<tr>
<td>Creatinine</td>
<td>μmol/L</td>
<td>44.2–114.92</td>
<td>171.49</td>
<td>180.34</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>μmol/L</td>
<td>3.42–17.10</td>
<td>117.31</td>
<td>142.61</td>
</tr>
<tr>
<td>AST</td>
<td>U/L</td>
<td>0–15</td>
<td>86.63</td>
<td>61.31</td>
</tr>
<tr>
<td>ALT</td>
<td>U/L</td>
<td>0–19</td>
<td>41.07</td>
<td>33.53</td>
</tr>
<tr>
<td>aPh</td>
<td>U/L</td>
<td>60–170</td>
<td>41.59</td>
<td>15.74</td>
</tr>
</tbody>
</table>

*AST = aspartate aminotransferase; ALT = alanine aminotransferase; aPh = alkaline phosphatase.

Data are expressed as mean and standard deviation of the mean or median and range for not normally distributed data. Spearman’s correlation coefficients were used for correlation analysis. Probability values of $P < 0.05$ were considered statistically significant.

RESULTS

Twenty-seven patients (four females and 23 males) could be evaluated. Their ages ranged from 16 to 45 years (mean ± SD = 24.8 ± 8.3). The mean ± SD temperature before treatment was 38.2 ± 1.1°C, the leukocyte count was 9.1 ± 4.4 × 10⁹/L, and the thrombocyte count was 68.9 ± 52.5 × 10³/L. Other routine laboratory parameters are shown in Table 1.

Parasite counts on admission ranged from 533 to 1,147,040 parasites/µL, with a median of 290,680 parasites/µL. With treatment, parasite counts decreased to 0 in all patients by day 7 and stayed negative on day 14. Eight patients experienced a recrudescence on day 21 but cleared their parasites again by day 28. The mean ± SD parasite clearance time (time to first negative blood film) was 59.3 ± 18.8 hr.

On admission, all but one patient had elevated ProCT levels. The level in untreated patients was 40 ng/ml (range = 0.04–662). The one patient with normal admission ProCT levels had a very low parasite count of only 533 despite a temperature up to 39°C before treatment.

On day 7, parasite counts in all patients were 0. Levels of ProCT at this time point were 1.3 ng/ml (range = 0.01–6.5, Figure 1). With the exception of patient 24, ProCT levels were within the normal range thereafter: day 14, 0.08 ng/ml (range = 0.01–20); day 21, 0.06 ng/ml (range = 0.01–1.5); day 28, 0.05 ng/ml (range = 0.01–1.4). Mean admission ProCT levels in eight patients with recrudescence were 185.3 ng/ml compared with 52.4 ng/ml in the remaining patients. The median ProCT level in healthy controls was 0.08 ng/ml (range = 0.04–0.9).

There was a significant correlation between initial parasite count and ProCT levels before treatment ($r = 0.43, P < 0.05$; Figure 2). No correlation between ProCT and routine laboratory parameters (leukocytes, thrombocytes, creatinine, liver enzymes) could be calculated.

Mean nitrite/nitrate levels on admission were 12.7 nmol/L (range = 1.0–35). There was a significant correlation between the serum nitrate and ProCT levels (Figure 3), as well as between nitric oxide (NO) and parasite clearance ($r = 0.59, P < 0.05$). In the group of healthy volunteers NO levels were < 1 nmol/L in all samples.

DISCUSSION

In healthy subjects, only minute amounts of ProCT appear in the serum. However, in subjects with severe bacterial infections, especially sepsis and peritonitis, elevated serum levels have been described.

While increased levels of ProCT are produced mainly in bacterial infections and have been used for differentiating bacterial from viral meningoencephalitis in infants, some protozoal and fungal diseases are also associated with high ProCT levels. These levels were elevated in all but one of our patients with complicated P. falciparum malaria. The serum levels on admission (i.e., before treatment) were as high as or even exceeded those measured in patients with septic shock.

There was a strong correlation between initial parasite counts and ProCT levels, and the only patient with a normal ProCT level on admission had the lowest parasitemia (533 parasites/µL). Initial parasitemia is an important clinical parameter for the severity of disease. While studies in Vietnamese patients and in Gabonese children also found high ProCT levels in individuals with malaria, no correlation with parasitemia was reported. However, these two studies also included patients with uncomplicated malaria and demonstrated significant differences in mean ProCT levels between the clinically defined groups. Because of the small number of deaths in the three studies, the predictive value of ProCT levels cannot be defined. Although in the study by Lenoble and others the one child with a ProCT level >100 ng/ml
died and in the study of patients with melioidosis by Smith and others\(^9\) levels of >100 ng/ml seem to be strongly predictive of a lethal outcome, none of our patients died despite ProCT levels up to 662 ng/ml.

Cytokines that have been found to be closely related to ProCT levels in clinical and experimental sepsis, such as IL-6 and TNF-\(\alpha\), are consistently elevated in severe \textit{P. falciparum} malaria. They seem to be closely correlated with disease severity and outcome although these findings are not unanimous.\(^{12-16}\) Since ProCT levels increase approximately 2 hr after TNF and IL-6 following experimental endotoxin injection and before C-reactive protein, a possible role in the acute-phase reaction seems likely. Up to now, however, neither the metabolic function nor the cellular source of ProCT has been elucidated.

Nitric oxide is part of the nonspecific host defense, a phenomenon involving the reticuloendothelial system, hepatocytes, and the endothelium. Its cytotoxicity increases the ability of macrophages to kill bacteria, viruses, and protozoa. Consistent with increased killing capacities, nitrite/nitrate levels measured in our patients correlated with parasite clearance time but not with initial parasite counts. Elevated levels of NO in \textit{P. falciparum} malaria have been reported to correlate with the severity of the disease.\(^{17}\) However, because of its cytotoxic potential that contributes to tissue injury, it has been postulated by Clark and others\(^{18}\) that reactive nitrogen intermediates play a major role in the development of cerebral malaria.

Levels of NO were increased in all of our patients with complicated \textit{P. falciparum} malaria for at least 21 days. In contrast to levels of ProCT, which decrease rapidly as the condition of the patient improves, NO also seems to be important in later phases of the infection. At the time of admission, there was a positive correlation between ProCT and

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**Figure 2.** Correlation of procalcitonin levels on admission with initial parasite count.

**Figure 3.** Procalcitonin levels and nitric oxide (NO) levels before treatment.
NO that was lost after ProCT levels decreased with treatment. In contrast to ProCT, NO showed a correlation with parasite clearance time.

Since most components of the cytokine cascade are excreted via the kidney, impairment of renal function might contribute to increased levels found in various disease states. However, there was no correlation of ProCT with renal function in our patients, indicating that the increased amounts of ProCT are not due to diminished excretion.

In conclusion, we found high levels of ProCT in P. falciparum malaria that correlated with parasite density and can serve as markers of disease severity. Based on higher initial levels in patients who later experienced recrudescence, ProCT might be of value in predicting disease course and outcome. However, the small number of patients with recrudescence infections does not allow for definitive conclusions to be formed.

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