CONCISE COMMUNICATION

Procalcitonin as a Parameter of Disease Severity and Risk of Mortality in Patients with *Plasmodium falciparum* Malaria

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The serum levels of procalcitonin (PCT) in *Plasmodium falciparum* malaria were evaluated for clinical significance in 66 nonimmune and semi-immune patients. Of the 66 patients, 36 had uncomplicated malaria, 24 had severe and complicated malaria, and 6 had fatal malaria (5 from previous studies). Pretreatment PCT concentrations were closely correlated with parasitemia. Concentrations were lowest in semi-immune patients with uncomplicated malaria, compared with those in nonimmune patients (geometric mean concentrations [GMCs], 1.07 and 2.37 ng/mL, respectively), and were highest in severe and complicated cases (GMC, 10.67 ng/mL; $P < .001$ among all subgroups). Six of 7 patients with PCT concentrations >25 ng/mL died. PCT concentrations decreased on day 2 of treatment in survivors but not in patients with fatal outcome. Thus, repeated PCT measurements may provide useful prognostic information, especially in medical centers that are not experienced in parasite density determination.

Plasmodium falciparum malaria is a markedly heterogeneous disease with a prognosis that can range from complete recovery to death. The clinical course of falciparum malaria may be punctuated by fatal complications. An important challenge for attending physicians in the management of falciparum malaria is to identify patients who are at high risk of complications and mortality and who may benefit from intensified care and treatment. Therefore, surrogate markers are needed to help identify such patients. Parasite count is the reference standard for this purpose; however, in countries where malaria is not endemic, many patients are diagnosed and treated at medical centers with little or no experience with the disease. Physicians and laboratory technicians may be confronted with a malaria patient only once during their working lives. Correct parasite counting is probably unreliable in these settings, and alternative parameters that can be determined easily in standard laboratories would be a valuable and desirable achievement.

Recently, there has been interest in the potential use of procalcitonin (PCT) as a biochemical marker of severity in different infectious diseases. Enthusiasm has been triggered by a nearly exponential increase of both retrospective and prospective studies that consistently have documented elevated serum concentrations of PCT in various severe bacterial or parasitic infections, such as sepsis [1], pyelonephritis [2], melioidosis, disseminated candidiasis, pneumonitis [3], and pulmonary tuberculosis [4]. Reports of elevated serum concentrations of PCT in patients with falciparum malaria have been of particular interest [5–8]. However, these studies lack sufficient information on adult and nonimmune patients and follow-up data. Therefore, we examined pretreatment and follow-up values of serum PCT concentrations in adult nonimmune and semi-immune patients with falciparum malaria and assessed the suitability of PCT as a surrogate marker of disease severity and mortality.

Patients and Methods

Patients. We enrolled 66 patients who were admitted to the Department of Medicine, Bernhard Nocht Institute for Tropical Medicine (Hamburg, Germany), with the diagnosis of imported falciparum malaria. Of these 66 patients, 61 were enrolled consecutively: 25 had severe and complicated falciparum malaria, including 1 fatal case, and 36 had uncomplicated falciparum malaria. To increase the number of fatal cases, we added 5 historical cases with fatal outcome for whom samples were available from previous studies [9–12]. Special care was taken to exclude patients with concomitant infections by evidence of history, clinical examination, and laboratory findings, to avoid a possible effect on PCT concentrations. Patients were defined as semi-immune if they were reared in a malarious area and had lived in a nonmalarious area for ≤ 5 years. Nonimmune patients were persons reared in nonmalarious areas who had lived in a malarious area for ≤ 2 years. Patients were considered to have severe and complicated falciparum malaria if they met ≥ 1 of the following criteria, as described elsewhere [9–12]: impaired cerebral function; pathologic global clotting tests (prothrombin time [PT] activity <50% and activated partial

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Written informed consent was obtained from all patients before study inclusion, in accordance with guidelines of the authors' institutions.

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thromboplastin time [aPTT] >45 s); impaired renal function (serum creatinine level >2.0 mg/dL); respiratory insufficiency (PO_2 <60 mm Hg); hepatic damage (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) levels ⩾100 U/L); and >5% parasitemia. Blood samples were drawn immediately before treatment (day 0) and on days 1, 2, 4, and 7 after commencing treatment. Serum samples were separated by centrifugation and immediately were stored at −80°C until analysis in a batch.

Medical care. Antimalarial therapy was started immediately after malaria diagnosis was established. Patients with severe and complicated falciparum malaria received standard doses of intravenous quinine and doxycycline. Those with uncomplicated falciparum malaria received standard doses of either mefloquine or halofantrine. The following routine tests were done before the administration of the first dose of antimalarial therapy and throughout the course of the disease: electrocardiography; chest radiography; a complete blood count with white blood cell differential and platelet and reticulocyte counts; hemoglobin concentration, coagulation tests of PT, aPTT, and fibrinogen plasma levels; serum levels of creatinine, ALT, AST, glucose, and lactate dehydrogenase (LDH); blood gas analysis; and fluid balance. Vital signs were recorded, and physical examinations were done at regular intervals throughout the study period. In particular, patients were examined for evidence of pulmonary edema, renal dysfunction, and impaired cerebral function (disorientation, drowsiness, and unconsciousness).

Laboratory investigations. Disease activity was evaluated clinically and parasitologically by monitoring of asexual parasite forms in peripheral blood subjected to Giemsa staining of thick blood film. Parasitemia was calculated from thick films and was reported as absolute number of parasites (number per microliter) by counting parasites against white blood cells, using the patient's known white blood cell count. Serum concentrations of PCT, neopterin (NPT), creatinine, and lactate dehydrogenase were determined from the same samples. PCT and NPT were determined by immunoluminometric assay (LUMItest PCT) and RIA, respectively, according to the manufacturer's specifications (BRAHMS Diagnostica).

Statistical analysis. We analyzed data with a commercial computer software package (SYSTAT version 9; SPSS). Differences among means were calculated by Student's *t* test, partly using log-transformed values. Correlation was computed by the Pearson test. Discriminant analysis was performed by complete, forward stepwise, and backward stepwise estimation. Significance was assumed at $P < .05$.

Results

Patient subgroups were well matched regarding sex, age, and duration of symptoms. Pretreatment serum PCT concentrations of patients with severe and complicated malaria were 2.61–132.1 ng/mL (geometric mean concentration [GMC], 10.67 ng/mL). In comparison, PCT concentrations in patients with uncomplicated malaria were 0.5–5.6 ng/mL (GMC, 1.52 ng/mL). Normal values are expected to be <0.5 ng/mL. There was a significant difference in PCT concentrations among semi-immune patients, nonimmune patients with uncomplicated malaria, and nonimmune patients with severe and complicated malaria (figure 1). PCT concentrations in both groups increased on day 1 and decreased on day 2. This pattern was not observed in fatal cases (figure 2).

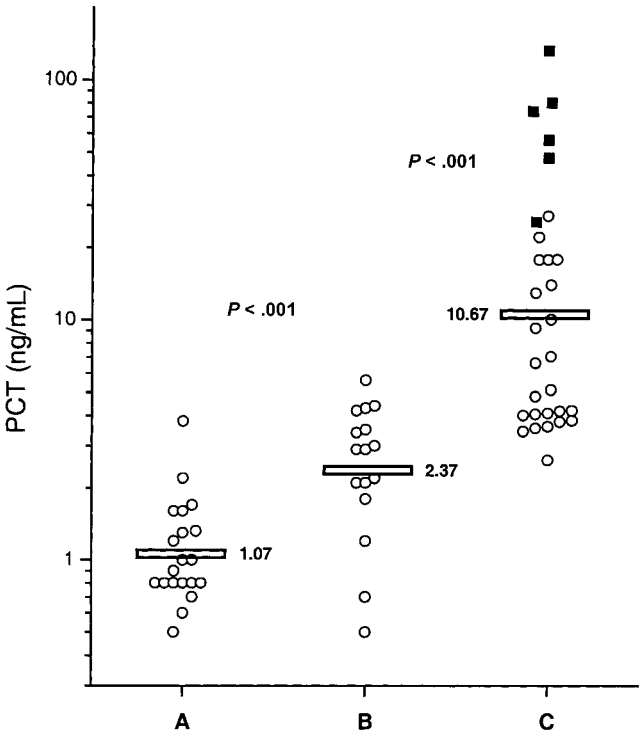

Figure 1. Pretreatment procalcitonin (PCT) serum concentrations in falciparum malaria patients. A, uncomplicated malaria in semi-immune patients ($n = 20$); B, uncomplicated malaria in nonimmune patients ($n = 16$); C, severe malaria ($n = 30$). *Horizontal bars* and *nos.*, geometric mean concentrations; ■, 6 patients who died. Differences between all subgroups are significant ($P < .001$).

Concentrations returned to near normal values on day 7. This was paralleled by clinical improvement and parasite clearance. A striking finding was that PCT concentrations were markedly higher in the 6 fatal cases (range, 25.63–132.11 ng/mL) and kept increasing after day 1 in 3 patients who survived until day 2. The increase of PCT concentration was associated with clinical deterioration and subsequent death.

Correlations of parasitemia with PCT, NPT, LDH, and creatinine levels were further analyzed. PCT was the only parameter that correlated significantly with parasitemia in nonimmune patients with uncomplicated malaria. In severe and complicated malaria, PCT showed the strongest correlation with parasitemia. Although LDH also showed a good correlation in this subgroup, a considerable number of patients showed LDH values within the normal range, whereas PCT concentrations were markedly elevated in all patients (figure 1).

We used discriminant analysis to test the discriminative contribution of log-transformed values of parasite count, PCT, NPT, and LDH, as well as creatinine levels. As a single parameter, parasite count performed best, as shown by the correct classification percentages and Wilks λ values. Discrimination among different subgroups (survivors, nonsurvivors, immune,

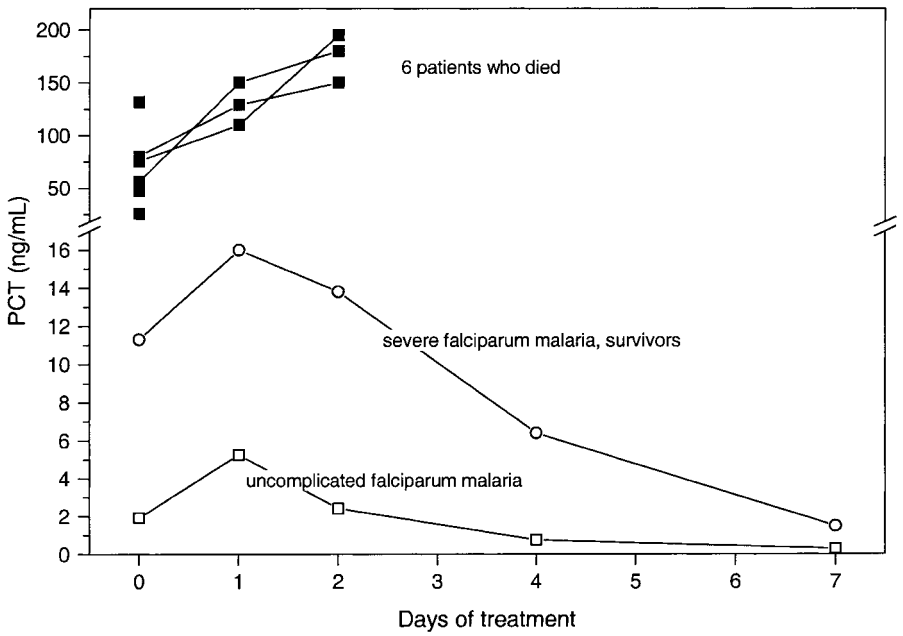

Figure 2. Follow-up values of procalcitonin (PCT) in 3 patient groups: uncomplicated falciparum malaria ($n = 36$); severe falciparum malaria, survivors ($n = 24$); and severe falciparum malaria, patients who died ($n = 6$). Pattern of declining PCT values on day 2 was seen in all surviving patients.

semi-immune, complicated, or uncomplicated), using PCT concentrations as a single parameter, came very close to parasite counts, with the exception of discriminating the immune status in uncomplicated cases. Regarding discrimination between survivors and nonsurvivors, PCT and parasite count as a single parameter correctly classified (Jackknife method) nonsurvivors in 100% and survivors in 90% and 83%, respectively. NPT showed good discrimination between survivors and nonsurvivors only in combination with at least LDH and creatinine levels. Altogether, there was only a minimal difference between parasite counts and PCT concentrations in all categories. However, NPT levels were unable to sufficiently discriminate patients with intermediate severity of disease. Of interest, the combination of parasite count with any of the other parameters resulted either in no significant improvement or in reduced discriminative power.

Discussion

Several clinical features and laboratory parameters are currently used to define and predict falciparum malaria disease severity [9–13]. Peripheral blood density of parasites is the single best parameter of disease severity and is regarded as the reference standard, although it may not adequately reflect the total number of parasites involved in the pathophysiologic process. A varying proportion of erythrocytes containing mature forms of falciparum parasites may sequester from the peripheral circulation by cytoadherence to capillary and postcapillary venular endothelium of vital organs [14, 15]. No acceptable alternative parameters to estimate disease severity and, of most importance, mortality are yet available for clinical centers not experienced in malaria diagnosis, accurate parasite counting, and medical care. Therefore, our study evaluated the significance of PCT, a parameter that could easily and repeatedly be determined in an average hospital laboratory.

Our results show that persons with severe and complicated falciparum malaria have significantly higher pretreatment PCT serum concentrations than do persons with uncomplicated malaria. In addition, the response to antimalarial therapy was reflected by a rapid decrease of PCT concentrations on day 2, coinciding with clinical improvement. The correlation between parasitemia and PCT concentrations was very high in severe and complicated falciparum malaria cases. In contrast to LDH, NPT, and creatinine levels, PCT levels in this subgroup were elevated in all patients and showed a high correlation with parasitemia, including nonimmune patients with uncomplicated falciparum malaria. An important clinical implication of our study is the high mortality risk of patients with pretreatment concentrations >25 ng/mL. The value of high PCT concentrations for predicting mortality should be further evaluated in a greater number of patients with fatal outcome.

Information on serum concentrations of PCT in malaria was gathered from studies of patients living in endemic areas who are continuously at risk of various infections. A major strength of this study was its composition of semi-immune and nonimmune patients without evidence of concomitant infections. This

excluded some possible contributing factors, apart from falciparum malaria, to elevated PCT concentrations. However, our study did not include children, an important group that is most susceptible to severe malaria. Serum concentrations of PCT may have been affected by when patients were seen at the hospital when blood samples were taken. Some patients may not have equated their symptoms with malaria after returning from malarious areas, so there is probably a variation in time from onset of infection to time of sampling. However, by multivariate analysis the duration of reported symptoms did not influence results.

In conclusion, our findings demonstrate that serum PCT concentrations are highly correlated with the absence of semi-immunity, the degree of disease severity, and mortality. PCT concentrations >25 mg/mL appear to indicate a highly increased risk of mortality. PCT may therefore serve as a suitable biochemical parameter in falciparum malaria, particularly in settings in which correct parasite counting is not easily done.

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