LOW LEVELS OF FIBRIN-STABILIZING FACTOR (FACTOR XIII) IN HUMAN PLASMODIUM FALCIPARUM MALARIA: CORRELATION WITH CLINICAL SEVERITY

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Abstract. Plasmodium falciparum malaria is associated with procoagulant activity but not with thromboembolism. We measured coagulation factor XIII, i.e., fibrin-stabilizing factor, in 45 patients with falciparum malaria over time. Of these, 22 had organ complications. The factor XIII antigen (subunits A and B) and plasma activity levels were abnormally low in those with falciparum malaria. They increased during antiparasitic therapy. In 14 of 22 patients with complications, but in no patient with mild disease ("P < 0.001), subunit A and activity was < 50%. The factor XIII levels were inversely correlated with clinical severity, parasitemia, and human neutrophil elastase (HNE), but not with thrombin-antithrombin III levels. Thus, low factor XIII levels may reflect proteolysis by HNE, rather than procoagulant activity. One could speculate that factor XIII degradation in severe malaria prevents thromboembolism. On the other hand, factor XIII deficiency might reduce protection of the vascular endothelium against HNE and reactive oxygen species, which would promote organ damage.

Human falciparum malaria can be complicated by potentially fatal organ failure. These complications tend to be associated with high parasitemia, distinct procoagulant alterations,13,14 and high serum levels of tumor necrosis factor-α (TNF-α).5 Tumor necrosis factor-α activates neutrophil granulocytes,5 which in turn may secrete lysosomal enzymes including human neutrophil elastase (HNE). These enzymes can increase the permeability of endothelial cell layers.5 In falciparum malaria, elevated HNE plasma levels correlate with high levels of circulating thrombomodulin,6 which suggests neutrophil-induced vascular damage in vivo. Increased capillary permeability may contribute to organ failure in severe malaria.9-11

In addition to inducing endothelial damage directly, HNE efficiently degrades coagulation factor XIII,12 which normally stabilizes fibrin clots by cross-linking fibrin monomers.13,14 Inactivation of factor XIII by HNE may contribute to hemostatic disorders and organ failure in gram-negative bacterial sepsis.15,16 as well as to vascular leakage in Purpura Schönlein-Henoch and inflammatory bowel diseases,17-19 in which factor XIII substitution may be beneficial. In guinea pigs, administration of factor XIII can protect from experimentally induced vascular damage.20

To assess the possible role of factor XIII in human malaria, we performed serial determinations of factor XIII plasma levels (antigen and activity) in 45 patients with falciparum malaria and analyzed how they were related to parasitemia, to the concentrations of TNFα and HNE, and to clinical severity.

PATIENTS AND METHODS

Patients. This study reports the results from 45 patients. Each patient had given consent to participate after receiving a full explanation of the study. Clearance had been obtained from the Ethics Committee of the Medical Board of Hamburg. All patients had contracted falciparum malaria while traveling in Africa. Except for eight African immigrants who had been living in Germany for at least two years, all patients were of European origin and had not been raised in malarious areas. Twenty-two of the 45 patients fulfilled one or more criteria for possible organ involvement (severe falciparum malaria) as listed in Table 1,2,5,8,21,22 which were used to identify high-risk patients prospectively. These 22 patients were treated with quinine and doxycycline for 10 days. Of the remaining 23 patients without signs of organ involvement (i.e., mild malaria), 11 received quinine plus doxycycline, and the other 12 were treated with mefloquine. Therapy was initiated as soon as falciparum malaria had been diagnosed by blood smear examination. All patients studied here survived without sequelae.

Blood sampling protocol. Blood was drawn into Sarstedt tubes (Sarstedt Corporation, Nümbrecht, Germany) after atraumatic puncture of the antecubital vein with a 20-gauge needle and no or minimal tourniquet pressure (up to 40 mm Hg) at the beginning of the procedure. Samples were drawn on days 0 (= before treatment), 1, 2, 4, and 7 of therapy. Serum and citrated plasma (platelet-poor plasma) were prepared immediately after blood sampling by centrifugation (2,000 × g) for 10 min at room temperature. The samples were aliquotted and stored at −80°C until analysis.

Analysis of patient samples. Parasitemia was calculated by counting the number of parasitized erythrocytes in 2,000 erythrocytes. The TNFα levels were determined by sandwich radioimmunoassay according to the instructions of the manufacturer (Medenix, Fleurus, Belgium). Coagulation factor XIII plasma activity was determined functionally as factor XIII-dependent incorporation of 125I-putrescine into ca-

### Table 1

<table>
<thead>
<tr>
<th>Criteria for severe disease: Plasmodium falciparum malaria was considered severe when one or more of the following criteria was presented (otherwise, it was considered mild malaria)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral involvement (drowsiness, disorientation, unconsciousness)</td>
</tr>
<tr>
<td>Renal involvement (creatinine &gt; 180 mmol/L)</td>
</tr>
<tr>
<td>Pathologic global clotting tests: PT &gt; 17 sec and PTT &gt; 45 sec</td>
</tr>
<tr>
<td>Respiratory failure (pO2 &lt; 8.6 kPa)</td>
</tr>
<tr>
<td>Liver involvement (ALT and/or AST &gt; 100 U/L)</td>
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</tbody>
</table>

* PT = prothrombin time; PTT = partial thromboplastin time; ALT = alanine aminotransferase; AST = aspartate aminotransferase.
sein.\textsuperscript{23} Factor XIII antigen concentrations (subunits A and B) were measured by Laurell electrophoresis.\textsuperscript{24} The HNE plasma concentrations were determined as its complex with α-1-protease-inhibitor with ELISA kits obtained from Merck (Harmstedt, Germany) (normal concentration < 90 ng/ml).\textsuperscript{25} Plasma concentrations of thrombin-antithrombin III (TAT) complexes were measured with a commercially obtained ELISA (Behringwerke, Marburg, Germany).\textsuperscript{26} With the exception of parasitemia, normal ranges for these parameters were verified by including samples from seven healthy European control subjects.

**Statistical analysis.** Nonparametric tests (Wilcoxon paired difference test and the Mann-Whitney U test) were used for significance testing. Correlations were calculated by the Spearman rank test. Any $P$ value $\leq 0.05$ (two-tailed, where applicable) was considered significant.

**Results**

**Parasitemia.** Before antiparasitic therapy, parasitemia ranged from 2.7 to 1,070 parasitized erythrocytes/ml of blood (median = 22.8) in severe falciparum malaria and from 2 to 148 parasitized erythrocytes/ml (median = 14.1) in mild falciparum malaria (Tables 1 and 2). Parasitemia was cleared at or before day 7 of therapy in all patients.

**Serum concentrations of TNF-α.** The TNFα levels were analyzed because this cytokine seems to play a role in malarial complications. As reported earlier,\textsuperscript{2,5} TNFα serum levels were elevated and correlated with parasitemia ($r = 0.071$, $P < 0.001$) upon admission (Table 3). They were higher in severe malaria than in mild falciparum malaria ($P < 0.001$; Table 2). During therapy, TNFα levels decreased towards normal values.

**Plasma levels of TAT.** The TAT plasma levels were determined as a parameter of procoagulant activity. Before therapy, elevated TAT levels (Table 2) correlated with parasitemia ($r = 0.39$, $P < 0.01$). The difference between severe and mild disease ($P < 0.05$) was less pronounced than in the case of TNFα. The TAT levels returned to normal values during antiparasitic treatment (Figure 1a).

**Human neutrophil elastase.** The HNE plasma levels were measured as a parameter of proteolytic activity. These levels were markedly elevated in untreated falciparum malaria. They were higher in severe cases than in uncomplicated cases ($P < 0.001$; Tables 1 and 2). During antiparasitic therapy, the elevation of HNE levels was reversed (Figure 1b). High HNE pretherapy levels were associated with high parasitemia ($r = 0.72$, $P < 0.0001$) and TNFα ($r = 0.78$, $P < 0.0001$). Thus, elevated HNE levels seem to be part of the host response in human malaria.

**Coagulation factor XIII.** The levels of factor XIII were determined since they are affected not only by procoagulant alterations, but also by proteolytic degradation.\textsuperscript{12} Before antiparasitic therapy, the plasma antigen concentrations of factor XIII subunits A and B (Figures 1c and 1d) were abnormally low in severe falciparum malaria (Table 1). They increased during antiparasitic therapy. Generally, the plasma concentrations of subunit A were lower than those of subunit B (Table 2). In contrast to severe disease, normal factor XIII activity levels and normal concentrations of factor XIII subunit B were observed in all cases of mild falciparum malaria before, during, and after therapy.

**Table 2.** Laboratory findings in untreated falciparum malaria*

<table>
<thead>
<tr>
<th></th>
<th>Normal values</th>
<th>All patients (n = 45)</th>
<th>Severe disease (n = 22)</th>
<th>Light disease (n = 23)</th>
<th>Significance (difference between severe and light disease)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasitemia</td>
<td>None</td>
<td>23 (2–1,070)</td>
<td>208 (2.7–1,070)</td>
<td>14 (2–148)</td>
<td><strong>P &lt; 0.01</strong></td>
</tr>
<tr>
<td>TNFα (pg/ml)</td>
<td>&lt;15</td>
<td>39 (&lt;15–896)</td>
<td>142 (35–896)</td>
<td>30 (&lt;15–108)</td>
<td><strong>P &lt; 0.001</strong></td>
</tr>
<tr>
<td>TAT (ng/ml)</td>
<td>&lt;5</td>
<td>8 (1.5–80)</td>
<td>12 (2.6–80)</td>
<td>7 (1.5–55)</td>
<td><strong>P &lt; 0.05</strong></td>
</tr>
<tr>
<td>HNE (ng/ml)</td>
<td>&lt;90</td>
<td>188 (26–4,845)</td>
<td>241 (102–4,845)</td>
<td>102 (26–357)</td>
<td><strong>P &lt; 0.001</strong></td>
</tr>
<tr>
<td>FXIIIa-Ag (%)</td>
<td>&gt;70</td>
<td>55 (7–108)</td>
<td>92 (50–108)</td>
<td>92 (50–108)</td>
<td><strong>P &lt; 0.001</strong></td>
</tr>
<tr>
<td>FXIIIb-Ag (%)</td>
<td>&gt;70</td>
<td>90 (32–130)</td>
<td>64 (32–100)</td>
<td>105 (76–130)</td>
<td><strong>P &lt; 0.001</strong></td>
</tr>
<tr>
<td>FXIII activity (%)</td>
<td>&gt;70</td>
<td>75 (23–111)</td>
<td>40 (23–85)</td>
<td>90 (70–111)</td>
<td><strong>P &lt; 0.001</strong></td>
</tr>
</tbody>
</table>

*Values are the median (range). Parasitemia = parasitized erythrocytes/ml of blood; TNFα = tumor necrosis factor α; TAT = thrombin-antithrombin III; HNE = human neutrophil elastase; FXIIIa-Ag = coagulation factor XIII antigen subunit α; FXIIIb-Ag = coagulation factor XIII antigen subunit β.

**Table 3.** Rank correlations between coagulation factor XIII levels and other parameters in human falciparum malaria (before therapy)*

<table>
<thead>
<tr>
<th></th>
<th>Parasitemia</th>
<th>TNFα</th>
<th>TAT</th>
<th>HNE</th>
<th>FXIIIb</th>
<th>FXIIIa</th>
</tr>
</thead>
<tbody>
<tr>
<td>FXIII activity</td>
<td>−0.44</td>
<td>−0.61</td>
<td>+0.04</td>
<td>−0.39</td>
<td>+0.74</td>
<td>+0.85</td>
</tr>
<tr>
<td>FXIIIa</td>
<td>−0.42</td>
<td>−0.52</td>
<td>−0.11</td>
<td>−0.39</td>
<td>+0.75</td>
<td>+0.85</td>
</tr>
<tr>
<td>FXIIIb</td>
<td>−0.43</td>
<td>−0.67</td>
<td>+0.11</td>
<td>−0.39</td>
<td>+0.75</td>
<td>+0.85</td>
</tr>
<tr>
<td>HNE</td>
<td>+0.72</td>
<td>+0.78</td>
<td>+0.58</td>
<td>+0.58</td>
<td>+0.75</td>
<td>+0.85</td>
</tr>
<tr>
<td>TAT</td>
<td>+0.39</td>
<td>+0.37</td>
<td>+0.37</td>
<td>+0.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNFα</td>
<td>+0.71</td>
<td>+0.71</td>
<td>+0.71</td>
<td>+0.71</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* TNFα = tumor necrosis factor α; TAT = thrombin-antithrombin III; HNE = human neutrophil elastase; FXIIIa = coagulation factor XIII subunit α; FXIIIb = coagulation factor XIII subunit β.

† $P < 0.01$.
‡ $P < 0.001$.
§ $P < 0.02$.
¶ $P < 0.0001$.
# $P < 0.005$.
** $P < 0.0002$.\textsuperscript{100 HOLST AND OTHERS}
Low factor XIII subunit A concentrations are correlated with high parasitemia \((r = -0.39, P < 0.02)\) and high HNE \((r = -0.39, P < 0.02; Figure 2a)\), but not with TAT levels \((r = -0.11, P > 0.10; Table 3)\). The same was true for factor XIII subunit B levels (parasitemia: \(r = -0.43, P < 0.01\); HNE: \(r = -0.39, P < 0.02\) [Figure 2b]; TAT: \(r = -0.11, P > 0.1\; Table 3)\).

The activity of factor XIII is associated with subunit A. Like subunit A antigen levels, factor XIII activity levels were abnormally low in untreated severe falciparum malaria.
FIGURE 2. Inverse correlation of plasma levels of coagulation factor XIII (FXIII subunit a and FXIII subunit b and FXIII activity; a–c) with those of human neutrophil elastase (HNE). In contrast, no such correlation was observed between FXIII and thrombin-antithrombin III (TAT) levels (d). Ag = antigen; falc. = falciparum.

(102) HOLST AND OTHERS

DISCUSSION

Previous studies have indicated that falciparum malaria is associated with normal factor XIII activity levels, and no significant change was observed during antiparasitic therapy. The difference between severe and mild falciparum malaria was significant (P < 0.001; Table 2). In addition, factor XIII activity levels correlated inversely with parasitemia (r = −0.44, P < 0.01; Table 3) and high HNE (r = −0.39, P < 0.02) levels (Figure 2c). No correlation between factor XIII and TAT levels could be observed (Table 3 and Figure 2d).

Low factor XIII levels probably do not reflect decreased synthesis of clotting factors. The function of the liver, which is the exclusive site of subunit B production, was not significantly impaired in the patients studied here, as shown by normal levels of coagulation factor IX. The only exception was one severely ill patient with liver involvement (alanine aminotransferase = 110 U/L, aspartate aminotransferase = 100 U/L, and coagulation factor IX = 40%) whose level of factor XIII subunit B (42%) was lower than his level of factor XIII subunit A (81%). Subunit A synthesis takes place mainly in cells related to the reticuloendothelial system and may be up-regulated by interferon-γ and TNFα. Both of these cytokines are part of the inflammatory response to falciparum malaria. Local up-regulation of factor XIII subunit A has been shown in other inflammatory diseases in which TNFα plays a critical role. These include rheumatoid arthritis and active Crohn’s disease, even though the plasma levels of factor XIII are abnormally low in the latter condition. For these reasons, low factor XIII plasma levels seem unlikely to reflect down-regulation of synthesis in diseases involving an inflammatory response (which includes malaria).

Although the response to malaria involves a procoagulant state, the abnormally low factor XIII levels found in this study may not reflect activation by thrombin and consumption because no correlation was observed between low factor XIII (antigen and activity) and elevated TAT levels. In addition, the concentrations of both subunits (A and B) were decreased in severe malaria, while procoagulant activity alone should only consume subunit A since subunit B is not...
a substrate for thrombin. Thus, thrombin formation is probably not the only cause of low factor XIII levels in malaria. Since both factor XIII subunits (A and B) are substrates for HNE, our findings are more compatible with proteolytic degradation of factor XIII than with procoagulant activity alone. The fact that subunit A (and factor XIII activity) levels were lower than subunit B levels (Table 2) is consistent with the in vitro observation that subunit A is more susceptible to proteolysis by HNE than subunit B. A pathogenic role of HNE-dependent proteolysis has already been discussed in complications of human gram-negative bacterial septicemia. In rats, inhibition of HNE protects from the effects of septic shock. In our study of falciparum malaria, we found higher HNE and lower factor XIII levels than those reported in bacterial sepsis by an earlier study. As high HNE and low factor XIII activity levels were associated with signs of organ failure (Tables 1 and 2). One possible mechanism could be proteolytic destruction of endothelial matrix proteins by HNE, which in turn would lead to disruption of the vascular endothelium and detachment of endothelial cells. In vitro, both HNE and HNE-secreting neutrophils can increase the permeability of endothelial cell layers. In vivo, this may contribute to vascular leakage and complications such as noncardiogenic lung edema, glomerular proteinuria, or inappropriate secretion of antidiuretic hormone, all of which have been described in severe falciparum malaria. This is consistent with another study in which severe disease and high HNE levels correlated with elevated plasma levels of thrombomodulin, a parameter reflecting endothelial damage.

Proteolytic cleavage of factor XIII may indirectly contribute to vessel wall and organ damage because factor XIII protects from experimentally induced vascular leakage and skin graft rejection in a mouse model, while inhibiting the potentially deleterious oxidative response of macrophages in vivo. Thus, besides stabilizing fibrin by cross-linking, factor XIII may play an important role in protecting the host from the potentially detrimental effects of a massive inflammatory response. Conversely, one might also speculate that proteolytic degradation of factor XIII by HNE possibly protects patients from thromboembolic events in the malaria-associated procoagulant state. However, no data support such a concept in human malaria. Future investigations should attempt to elucidate the role of factor XIII, HNE, and oxidative stress in endothelial damage and organ failure observed in falciparum malaria. The extent of HNE-dependent proteolysis in human malaria could be ascertained by measuring HNE-specific fibrinogen degradation products. It is hoped that a better understanding of the mechanisms discussed here may contribute to improved therapeutic strategies for falciparum malaria complicated by organ failure.

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REFERENCES

18. Suzuki R, Toda H, Takamura Y, 1989: Dynamics of blood co-