Severe complicated malaria caused by *Plasmodium falciparum* is a life-threatening condition. The symptoms include fever, metabolic acidosis, neurological impairment, circulatory collapse, and disorders of the coagulation system. The underlying pathogenesis is intriguing and comprises both a strong and complex inflammatory response and the sequestration of parasitized red blood cells (RBCs) in the microvasculature. In this study, we have measured the circulating levels of HMGB1 in 76 children with severe or uncomplicated malaria. Sera from both severe \((P = 0.0022)\) and uncomplicated \((P = 0.0049)\) patients had significantly higher circulating HMGB1 levels compared with healthy controls. Elevated HMGB1 in patients with ongoing *Plasmodium falciparum* infections might prolong inflammation and the febrile state of malaria and could offer a potential target for therapeutic intervention.

**Abstract.** Severe malaria is characterized by a massive release of proinflammatory cytokines in the context of sequestration of parasitized and normal red cells (RBCs). High-mobility group box 1 (HMGB1) is a DNA- and heparin-binding protein that also acts as a cytokine when released from cells in the extracellular milieu after a proinflammatory stimulus. In this study, we have measured the circulating levels of HMGB1 in 76 children with severe or uncomplicated malaria. Sera from both severe \((P = 0.0022)\) and uncomplicated \((P = 0.0049)\) patients had significantly higher circulating HMGB1 levels compared with healthy controls. Elevated HMGB1 in patients with ongoing *Plasmodium falciparum* infections might prolong inflammation and the febrile state of malaria and could offer a potential target for therapeutic intervention.

Severe complicated malaria caused by *Plasmodium falciparum* is a life-threatening condition. The symptoms include fever, metabolic acidosis, neurological impairment, circulatory collapse, and disorders of the coagulation system. The underlying pathogenesis is intriguing and comprises both a strong and complex inflammatory response and the sequestration of parasitized red blood cells (RBCs) in the microvasculature. There is compelling evidence that the inflammatory response, including the secretion of high levels of tumor necrosis factor (TNF), is of importance in bringing about the clinical manifestations of severe malaria infection—a disease state similar to bacterial sepsis.

High-mobility group box 1 (HMGB1) is a highly conserved protein with > 95% amino acid identity between the rodent and the human polypeptide. It is a member of the high-mobility group protein superfamily that has been widely studied as nuclear proteins that bind DNA, stabilize nucleosomes, and facilitate gene transcription. Albeit a nuclear protein involved in the regulation of transcription, HMGB1 is properly defined as a cytokine, because it stimulates proinflammatory responses in monocytes/macrophages, is produced during inflammatory responses in vivo in standardized models of systemic and local inflammation, mediates delayed endotoxin lethality, and is involved in downstream inflammatory responses in endotoxemia, arthritis, and sepsis. Furthermore, passive administration of anti-HMGB1 antibodies protects against experimentally lipopolysaccharide (LPS)-induced lethality, even when therapy is delayed after the proinflammatory cytokine response.

In this study, we have analyzed samples from children with uncomplicated or severe falciparum malaria with respect to the levels of HMGB1 in serum and found elevated levels in both groups. Serum samples were obtained from patients presenting with a primary diagnosis of malaria at Mulago Hospital in Kampala, Uganda. Ethical approval was obtained from the Uganda National Council for Science and Technology (MV 717) and Karolinska Institutet Regional Ethical Review Board (03/095).

Children (76 total), 51 children diagnosed with severe malaria and 25 children diagnosed with uncomplicated malaria, between 2 and 96 months (mean age = 27.5 months) were recruited after written informed consent of the guardian to participate in the study. Sera of healthy children could not be obtained because of ethical reasons. Control sera were, therefore, from healthy adult donors: 9 Ugandan and 12 Swedish donors. Patients with severe malaria were subgrouped according to clinical manifestations into cerebral malaria, malaria anemia, and severe cases non-ultra-descriptus (NUD) groups. HMGB1 serum levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Shino-Test, Kanagawa, Japan) according to manufacturer’s instructions. HMGB1 levels between different groups were compared using the Mann–Whitney U test.

**FIGURE 1.** Individual values and means of HMGB1 serum levels. HMGB1 serum levels measured in Swedish controls \((N = 12)\), Ugandan controls \((N = 9)\) and uncomplicated \((N = 25)\) and severe \((N = 51)\) malaria cases. Severe malaria was also subclassified into cerebral \((N = 16)\), anemia \((N = 13)\), severe NUD \((N = 13)\), and cerebral + anemia \((N = 9)\) malaria. Horizontal lines represent median values for each category. Dotted box indicate levels below the normal value of 1.4 ng/mL. Compared with controls, significant differences were found for all the groups: uncomplicated malaria \((P = 0.0017)\) and severe malaria \((P = 0.0004)\) patients had significantly higher circulating HMGB1 levels compared with healthy controls.
As seen in Figure 1, children suffering from malaria were found to have significantly higher serum levels of HMGB1 compared with the controls, both for the severe (P = 0.0022 compared with Ugandan and P = 0.0004 compared with Swedish controls) and uncomplicated cases (P = 0.0049 compared with Ugandan and P = 0.017 compared with Swedish controls). The interindividual levels varied (Figure 1), which has been seen previously in sepsis or influenza cases10,11 and may be explained by the fact that the duration of time since the onset of disease varied and therefore, the amount of HMGB1 released into circulation varied. HMGB1 levels in both healthy Ugandan and Swedish donors were low, mostly below the 1.4 ng/mL cutoff that denotes elevated HMGB112 (Figure 1).

In a previous study, it was found that HMGB1 levels in a small number of children who died of complicated cerebral malaria were much higher than the levels of healthy donors or of children with non-fatal cerebral malaria.13 Herein, we have analyzed a larger group of patients from a different geographical area and compared levels between different clinical conditions (Table 1). When analyzing different clinical manifestations of severe malaria, we could not detect any significant differences in the levels compared with the uncomplicated cases. Furthermore, no significant difference was detected when comparing different subgroups of severe malaria, but there was a trend in cerebral malaria patients to higher HMGB1 levels compared with children with anemia or other severe syndromes (Figure 1 and Table 1).

Our observations are in line with previous investigations of TNF and other cytokines implicated in the pathogenesis of severe malaria.6,14 Very high levels of TNF have been associated with severe and fatal disease.6 Our findings show that HMGB1 levels in children with malaria are significantly higher compared with controls and that the levels are in parity with those levels found during bacterial sepsis and other conditions of acute systemic inflammation.11,13,15,16 This result concurs with previous data.13 Furthermore, the levels of HMGB1 in patients with other diseases do not always correlate with severity or a lethal outcome.15,16

Passive administration of anti-HMGB1 antibodies protects against LPS-induced lethality in mice, even when therapy is delayed after the early proinflammatory cytokine response.19 The efficiency of delayed treatment of experimental sepsis with HMGB1 blockade up to 24 hours after its induction opens for the possibility to allow rescue from lethal human sepsis, and the same may be the case in severe, life-threatening malaria. Interestingly, HMGB1 is also a heparin-binding protein, and the binding may change the conformation of HMGB1 to reduce its affinity to receptor for advanced glycation end products (RAGE) and therefore, have an overall anti-inflammatory activity at low concentrations.20,21 Heparin is also known to block merozoite invasion and disrupt both rosettes and endothelial binding of pRBC, phenomena that are central to the pathogenesis of severe malaria.22

### Table 1

<table>
<thead>
<tr>
<th>Uncomplicated malaria (n = 25)</th>
<th>Cerebral malaria (n = 16)*</th>
<th>Malaria anemia (n = 13)*</th>
<th>Severe NUD malaria (n = 13)*</th>
<th>Cerebral malaria + anemia (n = 9)*</th>
<th>Severe malaria (n = 51)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMGB1 (ng/mL)</td>
<td>15.53 (2.5–33.2)</td>
<td>11.28 (2–15)</td>
<td>3.43 (2.3–10.3)</td>
<td>3.61 (1.3–15.5)</td>
<td>12.67 (5.6–18.4)</td>
</tr>
<tr>
<td>Age (months)</td>
<td>19 (12–36)</td>
<td>24 (13.5–36)</td>
<td>18 (12–24)</td>
<td>24 (12–48)</td>
<td>18 (12–42)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>38.9 (37.8–39.0)</td>
<td>39.9 (39.1–40.0)</td>
<td>39 (38.2–39.5)</td>
<td>40 (38.75–40.2)</td>
<td>39.8 (38.4–40)</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>100 (100–121)</td>
<td>125 (116–146)</td>
<td>135 (127.5–145)</td>
<td>125 (116–137.5)</td>
<td>130 (140–150)</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.6 (5.3–5.9)</td>
<td>5.4 (4.8–5.8)</td>
<td>5 (4.5–5.4)</td>
<td>5.4 (4.9–5.6)</td>
<td>4.9 (4.25–5.4)</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>8.2 (5.6–11)</td>
<td>5.4 (4.8–5.8)</td>
<td>3.7 (3–4.7)</td>
<td>10 (6.9–10.4)</td>
<td>2.9 (1.7–4.9)</td>
</tr>
<tr>
<td>WBC (×10^3/mm^3)</td>
<td>9.1 × 10^3</td>
<td>9 × 10^3</td>
<td>11.7 × 10^3</td>
<td>9.4 × 10^3</td>
<td>17 × 10^3</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>69.7 (67–73.6)</td>
<td>71.1 (64.4–76.3)</td>
<td>68.9 (62.7–78.6)</td>
<td>68.6 (62.3–71.1)</td>
<td>62.6 (60.7–82.7)</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>34.3 (33.1–34.8)</td>
<td>34.6 (33.8–35.8)</td>
<td>35.8 (34.3–38)</td>
<td>35 (33.7–36.5)</td>
<td>34.4 (32.7–37.4)</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>19.7 (17.1–22.5)</td>
<td>17.2 (15.4–22.7)</td>
<td>20.9 (19.8–26.5)</td>
<td>16 (15.1–16.8)</td>
<td>21.3 (17.8–29.7)</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>46.2 (19.9–56.5)</td>
<td>64.5 (59.6–71)</td>
<td>39.55 (31.6–51.2)</td>
<td>46.8 (29.2–64.9)</td>
<td>47.2 (36.6–58.4)</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>26.5 (16.8–31.2)</td>
<td>25.6 (21.1–31.6)</td>
<td>12.25 (8.9–15.2)</td>
<td>31.5 (26.3–33.4)</td>
<td>13.35 (8.4–24)</td>
</tr>
<tr>
<td>Platelets (×10^3/mm^3)</td>
<td>15 × 10^4</td>
<td>12 × 10^4</td>
<td>12 × 10^4</td>
<td>9 × 10^4</td>
<td>10 × 10^4</td>
</tr>
</tbody>
</table>
| Data are median (interquartile range). Hb = hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; RDW = red cell distribution width; WBC = white blood cells.

* Cerebral malaria, malaria anemia, severe NUD malaria, and cerebral malaria + anemia are subcategories of the severe malaria group.
REFERENCES


