Short Report: Elevated Levels of High-Mobility Group Box-1 (HMGB1) in Patients with Severe or Uncomplicated *Plasmodium falciparum* Malaria

Davide Angeletti, Mpungu Steven Kiwuwa, Justus Byarugaba, Fred Kironde, and Mats Wahlgren*

Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden; Department of Pediatrics, School of Medicine, Makerere University College of Health Sciences, Kampala, Uganda; Department of Biochemistry, School of Biomedical Sciences, Makerere University College of Health Sciences, Kampala, Uganda

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.¹ The symptoms include fever, metabolic acidosis, neurological impairment, circulatory collapse, and disorders of the coagulation system.² The underlying pathogenesis is intriguing and comprises both a strong and complex inflammatory response and the sequestration of parasitized and normal red blood cells (RBCs) in the microvasculature.^{3,4} 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.^{5,6}

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.⁷ 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.^{8–10} Furthermore, passive administration of anti-HMGB1 antibodies protects against experimentally lipopolysaccharide (LPS)-induced lethality, even when therapy is delayed after the proinflammatory cytokine response.¹⁰

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.¹² Compared with controls, significant differences were found for all the groups: uncomplicated malaria ($P = 0.017$ to Ugandan and $P = 0.0049$ to Swedish), severe malaria ($P = 0.0004$ to Ugandan and $P = 0.0022$ to Swedish), cerebral malaria ($P = 0.0087$ to Ugandan and $P = 0.0219$ to Swedish), malaria anemia ($P = 0.0021$ to Ugandan and $P = 0.005$ to Swedish), severe NUD malaria ($P = 0.0155$ to Ugandan and $P = 0.0276$ to Swedish), and cerebral malaria + anemia ($P = 0.0025$ to Ugandan and $P = 0.004$ to Swedish).

*Address correspondence to Mats Wahlgren, Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Box 280, Nobels vag 16, SE-171 77 Stockholm, Sweden. E-mail: mats.wahlgren@ki.se

TABLE 1
HMGB1 levels and clinical parameters of the patients included in the study

	Uncomplicated malaria (n = 25)	Cerebral malaria (n = 16)*	Malaria anemia (n = 13)*	Severe NUD malaria (n = 13)*	Cerebral malaria + anemia (n = 9)*	Severe malaria (n = 51)*
HMGB1 (ng/mL)	15.53 (2.5–33.2)	11.28 (2–15)	3.43 (2.3–10.3)	3.61 (1.3–15.5)	12.67 (5.6–18.4)	8.12 (2.4–14.3)
Age (months)	19 (12–36)	24 (13.5–36)	18 (12–24)	24 (12–48)	18 (12–42)	23 (12–36)
Temperature (°C)	38.9 (37.8–39.0)	39.9 (39.1–40)	39 (38.2–39.5)	40 (38.75–40.2)	39.8 (38.4–40)	39.55 (38.5–40)
Heart rate (bpm)	100 (100–121)	125 (116–146)	135 (127.5–145)	125 (116–137.5)	150 (140–150)	130 (122.5–145)
Glucose (mmol/L)	5.6 (5.3–5.9)	5.4 (4.8–5.8)	5 (4.5–5.45)	5.4 (4.9–5.6)	4.9 (4.25–5.4)	5.4 (4.7–5.6)
Hb (g/dL)	8.2 (5.6–11)	5.4 (4.8–5.8)	3.7 (3–4.7)	10 (6.9–10.4)	2.9 (1.7–4.9)	6.4 (3.8–10)
WBC (/mm ³)	9.1 × 10 ³ (6.3 × 10 ³ – 13.6 × 10 ³)	9 × 10 ³ (5 × 10 ³ – 10 × 10 ³)	11.7 × 10 ³ (7.8 × 10 ³ – 18 × 10 ³)	9.4 × 10 ³ (6.3 × 10 ³ – 14 × 10 ³)	17 × 10 ³ (8.4 × 10 ³ – 25.5 × 10 ³)	6.9 × 10 ³ (10 × 10 ³ – 17.7 × 10 ³)
MCV (fL)	69.7 (67–73.6)	71.1 (64.4–76.3)	68.9 (62.7–78.6)	68.6 (62.3–77.1)	63.6 (60.7–82.7)	68.6 (62.8–76.5)
MCHC (g/dL)	34.3 (33.1–34.8)	34.6 (33.8–35.38)	35.8 (34.3–38)	35 (33.7–36.3)	34.4 (32.7–37.8)	35.1 (33.6–36.5)
RDW (%)	19.7 (17.1–22.5)	17.2 (15.4–22.7)	20.9 (19.8–26.5)	16 (15.1–16.8)	21.3 (17.8–29.7)	18.7 (16–21.8)
Neutrophils (%)	42.6 (19.9–56.5)	64.5 (59.6–71)	39.55 (31.6–51.2)	46.8 (29.2–64.9)	47.2 (36.6–58.4)	49.9 (32.7–62.7)
Hematocrit (%)	26.5 (16.8–31.2)	25.6 (21.1–31.6)	12.25 (8.9–15.2)	31.5 (26.3–33.4)	13.35 (8.4–24)	17.55 (12–30.4)
Platelets (/mm ³)	15 × 10 ⁴ (10 × 10 ⁴ – 26 × 10 ⁴)	12 × 10 ⁴ (7.6 × 10 ⁴ – 24 × 10 ⁴)	12 × 10 ⁴ (7.5 × 10 ⁴ – 17.4 × 10 ⁴)	9 × 10 ⁴ (7.3 × 10 ⁴ – 21.8 × 10 ⁴)	10 × 10 ⁴ (3.8 × 10 ⁴ – 21.9 × 10 ⁴)	12 × 10 ⁴ (7.5 × 10 ⁴ – 18.8 × 10 ⁴)

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 subclassifications of the severe malaria group.

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 cases^{10,11} 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.^{15–19}

Passive administration of anti-HMGB1 antibodies protects against LPS-induced lethality in mice, even when therapy is delayed after the early proinflammatory cytokine response.¹⁰

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.^{22–24} Furthermore, heparin of a low-anticoagulant activity (Sevuparin, Dilaforette AB, Stockholm, Sweden) has been shown to dislodge sequestered pRBC into circulation and effectively disrupt rosettes of fresh clinical isolates.²⁵ The use of Sevuparin as adjunct therapy in severe malaria could, therefore, be beneficial not only because of its antiadhesive properties but also, its HMGB1 binding capacity to reduce inflammation and diminish the overall clinical manifestations.

In conclusion, our data support the notion that malaria is, at least in part, an inflammatory disease similar to other severe infections, such as septicemia. HMGB1 may play an important role in both disease progression and the febrile states, possibly prolonging the symptoms of the disease. HMGB1 should be considered as a target for treatment in severe malaria.

Received August 29, 2012. Accepted for publication November 30, 2012.

Published online February 11, 2013.

Acknowledgments: The authors thank the children and parents who volunteered to participate in the original trial, the investigators and staff at Mulago Hospital in Kampala, the research staff at the Med Biotech Laboratories, Kampala, Uganda, and Dr. Johan Normark, Ulf Ribacke, and Judy Orikiriza for sample collection. Ethical approval was obtained from the Uganda National Council for Science and Technology (MV 717) and Karolinska Institutet Regional Ethical Review Board (03/095).

Grants: This work was supported by Swedish Research Council (Vetenskapsrådet, VR) Grant VR/2012-2014/521-2011-3377, the Swedish Academy of Sciences (Kungliga Vetenskaps Akademien

[KVA]; Söderberg Foundation), Karolinska Institutet—Distinguished Professor Award (DPA), and the European Union Network of Excellence EviMalar.

Authors' addresses: Davide Angeletti and Mats Wahlgren, Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden, E-mails: Davide.angeletti@ki.se and Mats.wahlgren@ki.se. Mpungu Steven Kiwuwa and Justus Byarugaba, Makerere University College of Health Sciences, School of Medicine, Department of Pediatrics, Kampala, Uganda, E-mails: mkiwuwa@yahoo.com and byarugabaj@yahoo.com. Fred Kironde, Makerere University College of Health Sciences, School of Biomedical Sciences, Department of Biochemistry, Kampala, Uganda, E-mail: kironde@starcom.co.ug.

REFERENCES

1. World Health Organization, 2008. *World Malaria Report 2008*. Geneva: World Health Organization.
2. Maitland K, Marsh K, 2004. Pathophysiology of severe malaria in children. *Acta Trop* 90: 131–140.
3. Carlson J, Helmby H, Hill AV, Brewster D, Greenwood BM, Wahlgren M, 1990. Human cerebral malaria: association with erythrocyte rosetting and lack of anti-rosetting antibodies. *Lancet* 336: 1457–1460.
4. Newton CR, Warn PA, Winstanley PA, Peshu N, Snow RW, Pasvol G, Marsh K, 1997. Severe anaemia in children living in a malaria endemic area of Kenya. *Trop Med Int Health* 2: 165–178.
5. Clark IA, Virelizier JL, Carswell EA, Wood PR, 1981. Possible importance of macrophage-derived mediators in acute malaria. *Infect Immun* 32: 1058–1066.
6. Kwiatkowski D, Hill AV, Sambou I, Twumasi P, Castracane J, Manogue KR, Cerami A, Brewster DR, Greenwood BM, 1990. TNF concentration in fatal cerebral, non-fatal cerebral, and uncomplicated *Plasmodium falciparum* malaria. *Lancet* 336: 1201–1204.
7. Ueda T, Yoshida M, 2010. HMGB proteins and transcriptional regulation. *Biochim Biophys Acta* 1799: 114–118.
8. Harris HE, Andersson U, Pisetsky DS, 2012. HMGB1: a multifunctional alarmin driving autoimmune and inflammatory disease. *Nature Reviews Rheumatology* 8: 195–202.
9. Treutiger CJ, Mullins GE, Johansson AS, Rouhiainen A, Rauvala HM, Erlandsson-Harris H, Andersson U, Yang H, Tracey KJ, Andersson J, Palmblad JE, 2003. High mobility group 1 B-box mediates activation of human endothelium. *J Intern Med* 254: 375–385.
10. Wang H, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J, Frazier A, Yang H, Ivanova S, Borovikova L, Manogue KR, Faist E, Abraham E, Andersson J, Andersson U, Molina PE, Abumrad NN, Sama A, Tracey KJ, 1999. HMG-1 as a late mediator of endotoxin lethality in mice. *Science* 285: 248–251.
11. Ito Y, Torii Y, Ohta R, Imai M, Hara S, Kawano Y, Matsubayashi T, Inui A, Yoshikawa T, Nishimura N, Ozaki T, Morishima T, Kimura H, 2011. Increased levels of cytokines and high-mobility group box 1 are associated with the development of severe pneumonia, but not acute encephalopathy, in 2009 H1N1 influenza-infected children. *Cytokine* 56: 180–187.
12. Gaini S, Pedersen SS, Koldkjaer OG, Pedersen C, Moller HJ, 2007. High mobility group box-1 protein in patients with suspected community-acquired infections and sepsis: a prospective study. *Crit Care* 11: R32.
13. Alleva LM, Yang H, Tracey KJ, Clark IA, 2005. High mobility group box 1 (HMGB1) protein: possible amplification signal in the pathogenesis of falciparum malaria. *Trans R Soc Trop Med Hyg* 99: 171–174.
14. John CC, Opika-Opoka R, Byarugaba J, Idro R, Boivin MJ, 2006. Low levels of RANTES are associated with mortality in children with cerebral malaria. *J Infect Dis* 194: 837–845.
15. Karlsson S, Pettila V, Tenhunen J, Laru-Sompa R, Hynninen M, Ruokonen E, 2008. HMGB1 as a predictor of organ dysfunction and outcome in patients with severe sepsis. *Intensive Care Med* 34: 1046–1053.
16. Sunden-Cullberg J, Norrby-Teglund A, Rouhiainen A, Rauvala H, Herman G, Tracey KJ, Lee ML, Andersson J, Tokics L, Treutiger CJ, 2005. Persistent elevation of high mobility group box-1 protein (HMGB1) in patients with severe sepsis and septic shock. *Crit Care Med* 33: 564–573.
17. Angus DC, Yang L, Kong L, Kellum JA, Delude RL, Tracey KJ, Weissfeld L, 2007. Circulating high-mobility group box 1 (HMGB1) concentrations are elevated in both uncomplicated pneumonia and pneumonia with severe sepsis. *Crit Care Med* 35: 1061–1067.
18. Gibot S, Massin F, Cravoisy A, Barraud D, Nace L, Levy B, Bollaert PE, 2007. High-mobility group box 1 protein plasma concentrations during septic shock. *Intensive Care Med* 33: 1347–1353.
19. Alleva LM, Budd AC, Clark IA, 2008. Systemic release of high mobility group box 1 protein during severe murine influenza. *J Immunol* 181: 1454–1459.
20. Ling Y, Yang ZY, Yin T, Li L, Yuan WW, Wu HS, Wang CY, 2011. Heparin changes the conformation of high-mobility group protein 1 and decreases its affinity toward receptor for advanced glycation endproducts *in vitro*. *Int Immunopharmacol* 11: 187–193.
21. Rao NV, Argyle B, Xu X, Reynolds PR, Walenga JM, Prechel M, Prestwich GD, MacArthur RB, Walters BB, Hoidal JR, Kennedy TP, 2010. Low anticoagulant heparin targets multiple sites of inflammation, suppresses heparin-induced thrombocytopenia, and inhibits interaction of RAGE with its ligands. *Am J Physiol Cell Physiol* 299: C97–C110.
22. Mercereau-Puijalon O, Guillotte M, Vigan-Womas I, 2008. Rosetting in *Plasmodium falciparum*: a cytoadherence phenotype with multiple actors. *Transfus Clin Biol* 15: 62–71.
23. Vogt AM, Barragan A, Chen Q, Kironde F, Spillmann D, Wahlgren M, 2003. Heparan sulfate on endothelial cells mediates the binding of *Plasmodium falciparum*-infected erythrocytes via the DBL1alpha domain of PfEMP1. *Blood* 101: 2405–2411.
24. Vogt AM, Pettersson F, Moll K, Jonsson C, Normark J, Ribacke U, Egwang TG, Ekre HP, Spillmann D, Chen Q, Wahlgren M, 2006. Release of sequestered malaria parasites upon injection of a glycosaminoglycan. *PLoS Pathog* 2: e100.
25. Leitgeb AM, Blomqvist K, Cho-Ngwa F, Samje M, Nde P, Titanji V, Wahlgren M, 2011. Low anticoagulant heparin disrupts *Plasmodium falciparum* rosettes in fresh clinical isolates. *Am J Trop Med Hyg* 84: 390–396.