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# Comparison of parasite sequestration in uncomplicated and severe childhood *Plasmodium falciparum* malaria☆

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**Summary** *Objectives:* To determine whether sequestration of parasitized red blood cells differs between children with uncomplicated and severe *Plasmodium falciparum* malaria.
*Methods:* We quantified circulating-, total- and sequestered-parasite biomass, using a mathematical model based on plasma concentration of *P. falciparum* histidine rich protein 2, in Gambian children with severe ($n = 127$) and uncomplicated ($n = 169$) malaria.
*Results:* Circulating- and total-, but not sequestered-, parasite biomass estimates were significantly greater in children with severe malaria than in those with uncomplicated malaria. Sequestered biomass estimates in children with hyperlactataemia or prostration were similar to those in uncomplicated malaria, whereas sequestered biomass was higher in patients with severe anaemia, and showed a trend to higher values in cerebral malaria and fatal cases. Blood lactate concentration correlated with circulating- and total-, but not sequestered parasite biomass. These findings were robust after controlling for age, prior antimalarial treatment and clonality of infection, and over a realistic range of variation in model parameters.



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*Conclusion:* Extensive sequestration is not a uniform requirement for severe paediatric malaria. The pathophysiology of hyperlactataemia and prostration appears to be unrelated to sequestered parasite biomass. Different mechanisms may underlie different severe malaria syndromes, and different therapeutic strategies may be required to improve survival.
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## Introduction

Malaria causes around 1 million deaths per year globally.[1] Clinical features identify those at highest risk of death,[2,3] but even with appropriate antimalarial therapy, mortality rates remain at least 10–15%, and most deaths occur within 24–48 h of admission.[4,5] The pathophysiology of severe malaria is poorly understood, and hence the most appropriate supportive care strategies are largely unknown,[6–9] and effective adjunctive treatments are lacking.[10] Better understanding of the pathophysiology of severe malaria might direct better use of simple supportive treatments and reduce the huge burden of death.[11]

Most deaths from malaria occur in African children.[1] Paediatric severe malaria (SM) comprises several different, sometimes overlapping, syndromes – cerebral malaria (CM), severe anaemia (SA), hyperlactataemia (LA) (or a similar syndrome defined by acidosis or respiratory distress[11,12]) and severe prostration (SP).[13] CM and LA are common and associated with high risk of death.[2,14–16] The factors that determine why a child develops one rather than another SM syndrome are unknown. Parasitized red blood cells (pRBC) containing mature forms of *Plasmodium falciparum* adhere to vascular endothelium, a phenomenon known as sequestration,[17] and can cause microvascular obstruction, proposed to be central to the pathogenesis of SM.[11,18,19] Numerous sequestered pRBCs are found in the cerebral microvasculature of children and adults dying from CM,[20,21] and correlate with retinal microvascular pathology prior to death.[21] However, there are no contemporary postmortem studies in severe non-CM syndromes in children, and interpretation of data from postmortem studies is constrained by the absence of control groups with uncomplicated malaria (UM) (who, by definition, survive). Dondorp et al. estimated sequestered-parasite biomass from the plasma concentration of *P. falciparum* histidine rich protein 2 (PfHRP2).[22] Thai adults with SM had 10-fold higher sequestered-parasite biomass than those with UM,[22] but the association of sequestration with discrete SM syndromes was not examined. Other observations suggest mechanisms independent of pRBC sequestration may also contribute to SM: *Plasmodium vivax* can cause SM but exhibits little cyto-adherence[23,24]; even in fatal *P. falciparum* CM the degree of sequestration in cerebral vessels and tissues is extremely variable[21,25]; and soluble mediators can also cause endothelial dysfunction and microcirculatory impairment in SM.[11,26] Surprisingly, pRBC sequestration has never been compared between children with SM and UM controls, despite differences in SM manifestations between children and adults.[13,27] In the present study we aimed to quantify sequestered-parasite biomass in children with UM and SM.

## Methods

### Recruitment

With approval from the Gambia Government/MRC Laboratories Joint Ethics Committee, and the Ethics Committee of the London School of Hygiene and Tropical Medicine, all samples were collected with informed consent from the child's parent or guardian and used for an unmatched case-control study nested within a larger prospective cohort, of which methodological details have been published.[28] During each malaria season from August 2007 through January 2011, all Gambian children (<16 years old) presenting to any of three health centres with *P. falciparum* malaria (defined by clinical symptoms and ≥5000 asexual parasites/μL blood) were eligible for recruitment. Clinical management followed Gambian government guidelines, with SM cases admitted to hospital. Blood cultures were not routinely performed, but children were excluded if the attending clinician suspected concomitant bacterial infection. SM was defined using modified WHO criteria[13]: SA, hemoglobin <5 g/dL; LA, blood lactate >5 mmol/L; CM, Blantyre coma score ≤2 for at least 2 h in the absence of hypoglycemia; SP, inability to sit unsupported (children >6 months of age) or inability to suck (children ≤ 6 month). Children fulfilling criteria for both SP and SA, LA, or CM were classified as having SA, LA, or CM rather than SP. Eligible children without signs of SM were classified as UM. On presentation, capillary blood was used to measure lactate and glucose and to prepare thick and thin blood films; venous blood was collected for sickle cell screen, full blood count, and plasma storage (transported to the laboratory on ice within 2 h, separated and stored at −70 °C). Outcome was assessed by survival 7 days after presentation.

### PfHRP2 ELISA and parasite biomass calculation

PfHRP2 was measured in duplicate in plasma by ELISA kit (Cellabs) following the manufacturer's instructions with addition of a standard curve. Laboratory personnel were unaware of the clinical status of subjects. Circulating-, total-(PfHRP2-derived), and sequestered-parasite biomass estimates were calculated using formulas derived by Dondorp et al.[22] with an initial parasite replication rate of 7.5 (the average estimated in African children with SM),[29] an elimination constant of 1.26,[30] and modification of the blood volume term in the equation to improve accuracy for children as follows: males, blood volume (mL) = 312 + (63.11 × body weight (kg)); females, blood volume (mL) = 358 + (62.34 × body weight (kg)).[31] To account for variation in size of children, parasite biomass was expressed as parasites/kg body weight. Positive and negative values for sequestered biomass