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Stuck in a rut? Reconsidering the role of parasite sequestration in severe malaria syndromes

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Abstract

Severe malaria defines individuals at increased risk of death from their infection. Proposed pathogenic mechanisms include parasite sequestration, inflammation, and endothelial dysfunction. Severe malaria is not a single entity, manifesting with distinct syndromes such as severe anemia, severe respiratory distress or coma, each characterized by differences in epidemiology, underlying biology, and risk of death. The relative contribution of the various pathogenic mechanisms may differ between syndromes, and this is supported by accumulating evidence, which challenges sequestration as the initiating event. Here we propose that high parasite biomass is the common initiating feature, but subtle variations in the interaction between the host and parasite exist, and understanding these differences may be crucial to improve outcomes in patients with severe malaria.

Keywords

Severe malaria; sequestration; inflammation; endothelial activation; biomass

Severe malaria

Symptomatic malaria occurs during the erythrocytic stage (see Glossary) of infection with protozoal parasites of the genus *Plasmodium*, when parasites replicate within red blood cells (RBCs). The term severe malaria applies to manifestations associated with an increased risk of death or other adverse outcome (such as brain injury). Of the six *Plasmodium* species known to infect humans, *Plasmodium falciparum* causes the greatest burden of disease and accounts for up to 1.24 million deaths annually, mostly in African children [1]. The definition of severe malaria is based on World Health Organization criteria [2] and includes clinical and laboratory features, which are predictive of death in those receiving antimalarial treatment. However, the vast majority of children with severe malaria can be recognized from the presence of just three clinical syndromes: (i) coma (cerebral malaria); (ii) severe

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respiratory distress, characterized by fast and deep breathing; and (iii) severe anemia [3]. These syndromes can occur alone or in combination, and mortality rates differ between syndromes [3-5]. The combination of severe respiratory distress and cerebral malaria is associated with an additive increase in mortality whilst the presence of severe anemia may protect from the mortality of cerebral malaria [3]. There are clear differences in the epidemiological [6-7], clinical, and biological characteristics [8-15] of the three severe malaria syndromes (Table 1), which we believe indicate distinct underlying pathology. Recent evidence suggests that one important difference may be the extent of sequestration of parasitized RBCs [9]. Although effective antimalarial treatment can be lifesaving, case fatality rates remain high despite such treatment (Table 1) [16], and to date no adjunctive therapies have been proven to reduce this mortality [17]. Better understanding of the pathophysiology of severe malaria syndromes is crucially important to improving treatment.

What causes severe malaria?

The pathogenesis of severe malaria is hotly debated; some authors suggest that sequestration (Box 1) is the overriding pathogenic mechanism [17], whilst others believe that inflammatory processes are more important [18] (Figure 1A). An accumulating body of evidence indicates that vascular endothelial dysfunction is also important [15] and could be the interface between sequestration and inflammation [19]. Establishing the causal role of any single mechanism in severe malaria in humans is difficult, and no existing animal model reproduces all of the features of severe *Falciparum* malaria faithfully enough to satisfy all protagonists [20]. As discussed below, we believe that all three mechanisms are important and intrinsically linked, but their relative importance may vary between severe malaria syndromes.

Sequestration

Parasitized red blood cells (pRBCs) containing mature asexual stages of *P. falciparum* characteristically sequester in small blood vessels, doing so in such numbers that mechanical obstruction of blood flow can occur [17] (Figure 1A). The propensity of *P. falciparum* to sequester is assumed to explain why it is the most deadly of the malaria parasites infecting humans. Postmortem studies of subjects with cerebral malaria often show pRBCs obstructing small blood vessels (the microvasculature) in the brain [17, 21], and it is easy to imagine how this could account for the clinical manifestations of coma deteriorating to death. However, post mortem studies, by definition, do not include survivors with severe or uncomplicated malaria, and the extent of cerebral microvascular obstruction in non-fatal cases is unknown. Attempting to overcome this limitation, the accessible microvasculature of the retina [22] and rectal mucosa [23-24] has been visualized in live subjects, and abnormalities of blood flow are indeed observed; nevertheless, existing methods cannot discriminate whether these are actually caused by sequestered pRBCs. Post mortem studies show that microvascular sequestration also occurs in many tissues other than the brain [21], and it is assumed [24] (but not proven [25]) that this causes a sequence of impaired blood flow and oxygen delivery, a shift from aerobic to anaerobic glucose metabolism, generation of lactic acid, and ultimately a drop in blood pH (acidosis). Respiratory distress reflects the physiological response to metabolic acidosis, aiming to normalize blood pH by increasing respiratory excretion of carbon dioxide. As predicted, therefore, hyperlactatemia, acidosis, and respiratory distress are overlapping syndromes and have similar prognostic value [26], but there have been very few studies of their pathogenesis. We believe that sequestration alone fails to provide an adequate explanation for all features of severe malaria such as: (i) the typical rapid and complete recovery from coma and acidosis in most survivors after starting antimalarial therapy [27-28]; (ii) the rarity of infarction (areas of tissue death) [21],

despite the apparent blockage of blood vessels packed with pRBCs; and (iii) the occurrence of severe disease with *Plasmodium vivax*, which exhibits relatively little sequestration [29].

Immunopathology

An exuberant or dysregulated inflammatory response has long been a popular hypothesis for the pathogenesis of severe malaria [30], evolving with some analogy to sepsis [31], because numerous studies have demonstrated elevated concentrations or ratios of inflammatory mediators when compared with uncomplicated malaria (Table 1) [12-14, 32]. Immunopathology is proven to contribute to experimental cerebral malaria in mice [20] and is also an attractive explanation for how *P. vivax* may cause severe malaria because it elicits a much greater pro-inflammatory response per parasite than does *P. falciparum* [33]. Cytokines and other inflammatory mediators released from leukocytes might have direct effects on target organs [18, 34], combinatorial effects with other sensitizing molecules such as free heme [35], or indirect effects mediated through activation of the vascular endothelium (Figure 1) [15, 19, 36], mitochondrial dysfunction, or metabolic derangement [37-38]. Clinical trials of immunomodulatory agents have so far been unsuccessful in severe malaria, which has led some to question whether immunopathology has any role [17], but may simply reflect that it is too late to reverse the effects of inflammation once severe symptoms have developed. Unfortunately, proving that severe malaria is due to immunopathology is just as difficult as proving that it is due to sequestration, and perhaps more so because of overlapping networks of inflammatory mediators with considerable redundancy. These same reasons are believed to account for the failure of immunomodulatory adjunctive therapies to demonstrate effectiveness in the much larger number of clinical trials in bacterial sepsis [31, 34].

Endothelial dysfunction

Activation of the vascular endothelium (Figure 1A) is increasingly believed to be an important contributor to severe malaria. The vascular endothelium can be activated by inflammatory cytokines and material released from pRBCs such as hemoglobin, glycosylphosphatidylinositol, and histones [39-41], and by the adhesion of pRBCs [36]. In its quiescent state the vascular endothelium is an important regulator of microvascular blood flow, a repressor of the coagulation cascade, and a barrier to substances within the circulation [42]. Activation of the vascular endothelium can interfere with all these properties, causing perfusion abnormalities, coagulation, and vascular leak [42], all of which have been described to a greater or lesser extent in severe malaria [15]. Recent studies have highlighted abnormalities of both directly observed microvascular blood flow [23-24] and impaired functional responses to perturbation [28, 43-45], associated with reductions in nitric oxide bioavailability [28, 44]. In addition, accumulating evidence demonstrates activation of clotting mechanisms in the microvasculature [36] and impairment of barrier function [46-47], which might allow the direct interaction of cytokines or toxins with host tissues. Sequestration, inflammation, and endothelial activation are probably tightly linked; both inflammation and pRBC adhesion can cause vascular endothelial activation, whilst activation of vascular endothelium can enhance pRBC binding to receptors such as ICAM-1 [19], making it almost impossible to determine the primary, initiating event (Figure 1B). However, endothelial activation may be a more important initiating event in non-sequestering *P. vivax* infections [33], which can cause all manifestations of severe malaria in children [48].

Measuring sequestration: what lies below the ears of the hippopotamus?

Pathogen load is a fundamental determinant of the severity of infectious diseases, but the relationship between parasite load and severity of *P. falciparum* malaria has been enigmatic

because of sequestration. Using an old South African analogy [49], what is seen in the blood (the circulating parasite biomass) may just be the “ears of the hippopotamus” (Figure 2A); much of the total parasite biomass, which includes the biomass of sequestered parasites, is hidden from view. A major advance has been a method to estimate the total biomass of parasites in living subjects from the plasma concentration of *P. falciparum* histidine rich protein 2 (PfHRP2) [50]. The plasma concentration PfHRP2 is proportional to the total parasite biomass, and concentrations in severe malaria patients are much higher than those in uncomplicated malaria [8-9, 50-51]. Indeed, data from several of these studies indicates that the risk of severe malaria increases dramatically over a threshold level of total parasite biomass or PfHRP2 concentration [9, 52] (Figure 2B). However, some of the most interesting information is obtained when the circulating parasite biomass is calculated and subtracted from the total parasite biomass; this gives an estimate of the sequestered parasite biomass. Only two studies have considered sequestered parasite biomass in severe malaria relative to levels in uncomplicated malaria and their findings differ somewhat [9, 50]. In Thai adults, extensive sequestration occurred in most patients with severe malaria, but 64% of patients had cerebral malaria (often in combination with other severe manifestations), and the association of each syndrome with sequestration was not analysed independently [50]. By contrast, in Gambian children, we found that cerebral malaria and severe anemia were associated with the highest levels of sequestration, whereas subjects with hyperlactatemia had high circulating and total parasite biomass, but sequestered parasite biomass similar to that in uncomplicated cases [9]. This implies that the importance of sequestration may differ between severe malaria syndromes in children. In other words, a large hippopotamus can be dangerous, whether above or below the waterline (Figure 2C), but perhaps the hippopotamus above the water may cause harm differently than the hippopotamus lurking below the water.

Why do different severe malaria syndromes occur?

Differences in epidemiology and mortality between severe malaria syndromes have been known for a long time and have hinted at variation in the underlying biology [6-7, 53], but until recently few studies have directly investigated this. If sequestration was the main cause of severe malaria, high levels of sequestration would be expected in cases of cerebral malaria and hyperlactatemia but not in cases of severe anemia, which is assumed to result from lysis of RBCs and suppression of hematopoiesis [54]. However, severe anemia and cerebral malaria share the same pattern of very high levels of PfHRP2 [8-9], high sequestered biomass [9], and have recently been associated with expression of the same *var* gene encoding the DC-8 variant of PfEMP1 [10], which confers binding to the endothelial protein C receptor [55]. Conversely, the finding of relatively little sequestration [9] in children with hyperlactatemia might be more consistent with the recent observation that parasites isolated from children with respiratory distress are strongly associated with a rosetting parasite phenotype [11]. These observations indicate that, whilst sequestration and consequent microvascular obstruction remains a highly plausible mechanism contributing to cerebral malaria, we must look beyond sequestration to explain its distinction from hyperlactatemia and severe anemia.

If sequestration *per se* does not provide sufficient explanation for some severe malaria phenotypes, then perhaps host factors hold the key, and there is certainly evidence that the different syndromes are characterized by marked dissimilarities in the nature of the inflammatory response (Table 1). Respiratory distress appears to be the “most inflammatory” syndrome [12], whilst severe anemia is characterized by particularly low levels of certain cytokines [14], including the regulatory cytokine IL-10 [13], in what some consider a dysregulated immune response [54]. However, typically the magnitude of the inflammatory response has not been considered in relation to either the total parasite biomass or the duration of infection. The latter is difficult to measure in naturally occurring

infections in humans, but is probably a critical determinant of the extent of damage to the host, regardless of the exact pathological mechanism. Regulatory mechanisms are more likely to avert tissue damage in a gradually developing insult than in one which occurs rapidly – to continue the analogy, a charging hippopotamus is likely to present a greater danger than one slowly drifting downstream. Fortunately malaria leaves tracks that mark its progress, in the form of pigment (hemozoin) in phagocytic cells [54]. This crystalline polymer of heme, left over from digestion of hemoglobin by the parasite, is as indigestible to human phagocytes as it is to the parasite, and thus accumulates, giving a crude estimate of the cumulative parasite burden preceding presentation [56]. Hemozoin accumulation in monocytes is particularly characteristic of severe malarial anemia, which also usually has a longer duration of illness prior to hospitalization, suggesting that severe anemia has a more insidious onset than other forms of severe malaria. Furthermore, hemozoin can modulate the inflammatory response of host phagocytic cells [56], which may in turn suppress the production of new RBCs from the bone marrow and worsen anemia [54]. We speculate that in contrast to severe anemia, hyperlactatemia results from a particularly rapid rise in parasite biomass, independent of cytoadhesive sequestration, and leads to tissue hypoxia through inflammatory endothelial activation and possibly microvascular obstruction by RBC rosettes. Interestingly, recent evidence suggests that parasite multiplication rate could be linked to the host metabolic environment through the activity of the NAD-dependent parasite histone deacetylase, PfSir2a [57]. This exciting finding opens a new line of investigation, which might ultimately explain the links between parasite replication rate, biomass, sequestration, and severe disease manifestations.

Why is understanding the basis of severe malaria syndromes important?

Approximately 15% of children with respiratory distress or cerebral malaria die, even with appropriate antimalarial treatment [16]. Few supportive interventions have been tested in high quality clinical trials so that even the assumed benefit of a simple measure such as blood transfusion is largely based on observational data [58]. However, the need to rigorously evaluate interventions has been emphasized by the few well-conducted trials that found evidence of harm from simple, logical treatments [59-60]. Understanding the nuances of the mechanisms underlying different severe malaria syndromes may illuminate new treatment strategies and improve the use of existing measures. Unfortunately, clinical trials of adjunctive therapies have not routinely stratified patients by discrete severe malaria syndromes, which might have resulted in missed opportunities to identify subgroups in which there is benefit or harm.

Concluding remarks and future perspectives

Accumulating evidence about the pathogenesis of severe malaria makes it increasingly difficult to justify pRBC sequestration as the sole initiating pathogenic event. Undoubtedly sequestration is important; it contributes to the generation of a high total parasite biomass, and it is tightly linked with inflammation and endothelial dysfunction. However, we believe that it is the combination of these mechanisms, with a different balance in each syndrome, which best explains the occurrence of distinct severe malaria syndromes. Better understanding of the pathogenesis of severe malaria syndromes, and better treatments, will only come from studies that systematically classify subjects by the combination of syndromes with which they present (Box 2). In some cases this will mean conducting larger studies, with larger sample sizes, in order to analyze these subgroups independently. Both host and parasite factors, which may influence pathogenesis will need to be compared simultaneously between groups, taking care to account for differences in age, parasite biomass, and chronicity of infection. Application of genomic, proteomic, and metabolomic methods to these very carefully classified subgroups will help to identify hitherto unknown

mechanisms, which determine the roles, and interactions, of host and parasite in each manifestation of severe malaria. Investigation of mechanisms controlling parasite growth and biomass will be particularly important. Many of the tools to undertake such studies already exist, but non-invasive imaging methods to quantify the extent and distribution of parasite sequestration in living people are needed to properly understand the role of sequestration. Defining the biology that underlies each severe malaria syndrome will allow supportive and novel adjunctive treatments to be tailored to the situations in which they are most likely to be effective.

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Glossary

Acidosis	a reduction in blood pH.
Adjunctive therapy	treatments aiming to reduce risk of death or complications when added to conventional antimalarial and supportive treatments, for example, anti-inflammatory therapies.
Cerebral malaria	malaria parasitemia associated with coma, formally defined based on a coma score and exclusion of other reasons for coma.
Circulating biomass	the number of <i>Plasmodium</i> parasites estimated to be in a person's circulating blood.
Cytoadhesion	the binding of parasitized red blood cells to vascular endothelium and other cell types, which involves the interaction of host cell receptors and parasite ligands.

Endothelial protein C receptor	a receptor on vascular endothelium, which helps to activate the anticoagulant activity of protein C, and to mediate its anti-inflammatory effects.
Erythrocytic stage	the stage of the parasite life cycle within red blood cells.
Gametocyte	the sexual form of the <i>Plasmodium</i> parasite within the red blood cell, which is taken up by feeding mosquitoes and allows transmission of infection.
Glycosylphosphatidylinositol	a pro-inflammatory parasite-derived glycolipid.
Heme	an iron-porphyrin prosthetic group which can be liberated from the hemoglobin released during the destruction of red blood cells.
Hemozoin	a heme polymer formed by the parasite in the process of hemoglobin degradation.
Hyperlactatemia	abnormally high level of lactic acid in blood, usually defined as > 5mmol/L.
Intercellular adhesion molecule-1 (ICAM-1)	an inducible molecule expressed on the surface of vascular endothelium which mediates adhesion of leukocytes and parasitized red blood cells.
Nitric oxide	an important signaling and effector molecule with numerous effects including dilatation of blood vessels and promotion of quiescence of vascular endothelium.
Parasitized red blood cell (pRBC)	a red blood cell containing one or more <i>Plasmodium</i> parasites.
<i>Plasmodium falciparum</i> erythrocyte membrane protein 1 (PfEMP1)	the best characterized family of parasite proteins expressed on the surface of the host red blood cell.
<i>Plasmodium falciparum</i> histidine rich protein 2 (PfHRP2)	a parasite protein that is released predominantly when schizonts rupture to release daughter parasites.
Respiratory distress	increased rate and / or depth of breathing, often as a compensatory mechanism for acidosis.
Ring-stage	the immature asexual form of the <i>Plasmodium</i> parasite during the first half of its development within the red blood cell.
Rosetting	binding of parasitized red blood cells to other uninfected red blood cells.
Schizont	the most mature, multinucleated, asexual form of the <i>Plasmodium</i> parasite within the red blood cell, which will rupture to release daughter parasites that infect new red blood cells.
Sequestered biomass	the number of sequestered parasites estimated to be in a person's body (sequestered biomass = total biomass – circulating biomass).

Sequestration	retention of parasitized red blood cells in some blood vessels, thereby reducing the numbers circulating in the rest of the blood stream.
Severe malaria	a manifestation of malaria infection, which is associated with increased risk of death or lasting damage.
Severe malarial anemia	a severe reduction in circulating red blood cells or hemoglobin below 5g/dL.
Supportive therapy	treatment measures that aim to correct the physiological derangements caused by malaria, such as blood transfusion to correct anemia or intravenous glucose to treat low blood sugar levels.
Total biomass	the total number of <i>Plasmodium</i> parasites estimated to be in a person's body (total biomass = circulating biomass + sequestered biomass).
Trophozoite	asexual stage of the <i>Plasmodium</i> parasite within the red blood cell, which develops from the ring-stage and is characterized by acquisition of hemozoin.
Var gene family	A family of about 60 genes that code for PfEMP1; each parasite only expresses one <i>var</i> gene at any time.
Vascular endothelium	the monolayer of cells that forms the interface of the blood vessels with the circulating blood.

Box 1**Sequestration and cytoadhesion**

Sequestration is the retention of pRBCs in some parts of the vascular system, which effectively reduces their numbers in the rest of the circulating blood. It can result from the cytoadhesion of pRBCs to the luminal walls of small blood vessels [61], biomechanical properties of pRBCs (rigidity or aggregation with other RBCs) slowing their passage through small blood vessels [62-63], or retention of pRBCs in the slow open circulation of the spleen [64]. Sequestration is a characteristic feature of *P. falciparum* infections, where erythrocytes containing mature trophozoites, schizonts (asexual stage), and developing gametocytes (sexual stages) are rarely detected on blood films. In postmortem samples from some patients with severe malaria, sequestration of ring-stages can also be seen [21]. Sequestration of pRBC in the capillaries is believed to offer a survival advantage to the parasite, by avoiding the “filtering” function of the spleen [64]. Sequestration may facilitate replication of asexual parasites, extending the duration of the infection and increasing both the generation and survival of sexual (transmissible) stages [64-65]. Sequestration appears less prominent in *P. vivax* and *Plasmodium knowlesi* infections where mature asexual parasites are routinely detected in the blood [66-67] and has not been reported for *Plasmodium malariae* or *Plasmodium ovale* infections. Some rodent and non-human primate malaria species do cytoadhere and sequester [68-70], suggesting it is an ancient and relatively conserved trait.

Cytoadhesion is mediated by specific binding of parasite ligands on pRBCs to host receptors expressed on vascular endothelial cells [71], and may be facilitated by aggregation of platelets, fibrin, and other components of the coagulation system [72]. The best characterized adhesive ligands are members of the highly polymorphic *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) variant surface antigen (VSA) family, encoded by the var gene family [61]. PfEMP1 variants differ in their specificity for binding to host receptors. VSA expression can vary over the course of an infection due to sequential expression of different VSA family members, and consequently, the cytoadhesive phenotype may vary as infection progresses [73]. Some of the best characterized host receptors for cytoadhesion are CD36 and intercellular adhesion molecule-1 (ICAM-1, CD54) [61, 71], and recent findings have implicated the endothelial protein C receptor (EPCR) in severe malaria [55]. Expression of host receptors on the vascular endothelium varies between tissues and is a dynamic process, which changes dramatically in response to inflammation and endothelial activation [42]. ICAM-1 in particular is upregulated by inflammation, whereas CD36 is constitutively expressed, and EPCR appears downregulated by pRBC adhesion [36, 74-75].

Box 2**Outstanding questions**

- Will improved understanding of the pathogenesis of severe malaria lead to better treatments?
- Why does malaria cause both distinct and overlapping clinical manifestations?
- What are the most important pathogenic mechanisms in each severe malaria syndrome?
- What is the extent and location of sequestered parasites in individuals with different severe malaria syndromes, particularly those who survive malaria?
- Can parasite replication strategies other than sequestration allow a high biomass to be achieved?
- Do various supportive and adjunctive treatments have dissimilar effects in different severe malaria syndromes?

Highlights

- The concept that parasite sequestration *per se* initiates severe malaria is challenged
- We contend that high parasite biomass is the main determinant of severe malaria
- Explanations for different severe malaria syndromes are suggested
- Understanding severe malaria pathogenesis is proposed as key to better treatment

Figure 1. The pathogenesis of severe malaria

(A) Mechanisms believed to be important in the pathogenesis of severe malaria. Sequestration of cytoadherent parasitized red blood (pRBCs) cells on the vascular endothelium of small blood vessels can result in mechanical obstruction of blood flow and activation of the vascular endothelium. Molecules released from pRBCs and host cells can also cause sequestration-independent vascular endothelial activation and dysfunction, which results in impaired blood flow, localized activation of clotting pathways, and disruption of endothelial barrier function. Inflammatory mediators are amongst the molecules, which can cause endothelial activation, but may also contribute directly to organ dysfunction, cell death, mitochondrial dysfunction, and metabolic derangements. **(B)** Interdependence of pathogenic mechanisms. It is probable that all three pathogenic processes occur in parallel and could amplify each other with increasing parasite biomass. Differences in the relative contributions of these mechanisms may determine the clinical presentation of severe malaria.

Figure 2. The ears of the hippopotamus

(A) Circulating malaria parasites that can be detected in blood are like the “ears of the hippopotamus” visible above water. Sequestered parasites are comparable to the body of the hippopotamus, hidden from sight beneath the surface of the water, and can have considerable bulk. The size of the whole hippopotamus represents the total parasite biomass. (B) Relationship between total parasite biomass and likelihood of severe malaria (SM, cerebral malaria, severe anemia, or hyperlactatemia) (derived from [9]). (C) Representation of circulating, sequestered, and total parasite biomass estimates in different severe malaria syndromes. In uncomplicated malaria the total biomass is small, and a high proportion of parasites are circulating. In cerebral malaria and severe anemia, the total parasite burden is much larger, and a large proportion of the parasites are sequestered. In hyperlactatemia, the total parasite biomass is large, but the sequestered biomass is much smaller and the circulating biomass accordingly larger.

Table 1
Features of different severe malaria syndromes in children

Syndrome	Age Most Affected [6]	Relative risk with increasing age [7]	Relative risk with increasing exposure [7]	Mortality [3-5]	Parasite biomass [8-9]	Associated Parasite <i>var</i> gene expression or adhesion phenotype [10-11]	Characteristic inflammatory response [12-14]	Endothelial activation [15]
Severe anemia	Youngest children	Decreases	Increases	~1% Reduces risk of cerebral malaria death	High total biomass Sequestered biomass > circulating biomass	DC-8 and DC13 variants Presumed endothelial binding	Low IL-10 Low IL-1α Low IL-6 High TNFα:IL-10	Unknown
Respiratory distress	Young children	Constant	Constant	~15% Additive with cerebral malaria	High total biomass Circulating biomass > sequestered biomass	Red cell binding to form rosettes	High TNF-α High IL-10 High TN Fcγ:IL-10	Unknown
Cerebral Malaria	Older children	Increases	Decreases	~15% Additive with respiratory distress	High total biomass Sequestered biomass > circulating biomass	DC-8 and DC13 variants Presumed endothelial binding	High TNF-α High IL-10	High Endothelial activation and injury biomarkers

DC, Domain cassette; IL-, interleukin-; TNF-α, tumour necrosis factor-α