Published in final edited form as:

Br J Haematol. 2010 June ; 149(5): 711–721. doi:10.1111/j.1365-2141.2010.08147.x.

Clinical Predictors of Severe Malarial Anaemia in a Holoendemic *Plasmodium falciparum* Transmission Area

Enrico M. Novelli¹, James B. Hittner², Gregory C. Davenport^{3,4}, Collins Ouma^{4,5}, Tom Were⁴, Stephen Obaro⁶, Sandra Kaplan⁷, John M. Ong'echa^{4,8}, and Douglas J. Perkins^{4,8,*}

¹Division of Haematology/Oncology, Vascular Medicine Institute, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

²Department of Psychology, College of Charleston, Charleston, SC, USA

³Department of Infectious Diseases and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA

⁴University of New Mexico Laboratories of Parasitic and Viral Diseases, Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya

⁵Department of Biomedical Sciences and Technology, Maseno University, Maseno, Kenya

⁶Division of Infectious Diseases, Michigan State University, College of Human Medicine, Lansing, MI, USA

⁷Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

⁸Global and Geographic Medicine Program, Division of Infectious Diseases, University of New Mexico School of Medicine, Albuquerque, NM, USA

SUMMARY

Severe malarial anaemia (SMA) is a common complication of *Plasmodium falciparum* infections, resulting in mortality rates that may exceed 30% in paediatric populations residing in holoendemic transmission areas. One strategy for reducing the morbidity and mortality associated with SMA is to identify clinical predictors that can be readily recognized by caregivers for prompt therapeutic interventions. To determine clinical predictors of SMA, Kenyan children (3–36 mos., n=671) presenting with acute illness at a rural hospital in Siaya District were recruited. Demographic, clinical, laboratory and haematological parameters were measured upon enrolment. Since HIV-1 and bacteraemia promote reduced haemoglobin (Hb) concentrations, children with these infections were excluded from the analyses. Children with *P. falciparum* mono-infections (n=355) were stratified into three groups: uncomplicated malaria (Hb≥11.0 g/dL); non-SMA (6.0≤Hb<10.9), and SMA (Hb<6.0 g/dL). SMA was characterized by a younger age, monocytosis, thrombocytopaenia, reticulocytosis, reduced erythropoiesis, elevated pigment-containing monocytes (PCM), respiratory distress, conjunctival and palmar pallor, splenomegaly, signs of malnutrition, and protracted fever and emesis. Logistic regression analysis demonstrated that age, reticulocyte count, presence of PCM and conjunctival and palmar pallor were significant predictors of SMA. Recognition of these clinical signs in children residing in resource-poor settings may help guide the identification and management of SMA.

*Corresponding Author: Dr. Douglas J. Perkins, Director, Global and Geographic Medicine Program, University of New Mexico, Department of Internal Medicine, Division of Infectious Diseases, MSC10-5550, 1 University of New Mexico, Albuquerque, NM 87131-0001, phone: 505-272-6867, fax: 505-272-8441, DPerkins@salud.unm.edu, Director, Laboratory of Viral and Parasitic Diseases, University of New Mexico/KEMRI, Centre for Global Health Research, PO Box 1578, Kisumu, Kenya.

Keywords

Malaria; *Plasmodium falciparum*; anaemia; pallor; respiratory distress

INTRODUCTION

Greater than 80% of malaria-related mortality occurs in sub-Saharan Africa due to infections with *Plasmodium falciparum* (Berkley, *et al* 2005b, Snow, *et al* 1999b, WHO 2005). The majority of *P. falciparum*-related mortality occurs in immune-naïve infants and young children, accounting for 18% of all deaths before five years of age (Snow, *et al* 1999b, WHO 2005). Clinical manifestations of severe falciparum malaria vary according to transmission intensity and typically present as one or more life-threatening complications, including: hyperparasitaemia; hypoglycaemia; cerebral malaria (CM); severe malarial anaemia (SMA) and respiratory distress (Dzeing-Ella, *et al* 2005, English, *et al* 1998, Mockenhaupt, *et al* 2004). In holoendemic transmission areas, SMA is the primary clinical manifestation of severe childhood malaria, with CM occurring only rarely (Bloland, *et al* 1999, Ong'echa, *et al* 2006).

SMA is responsible for ~30% of the mortality in children less than three years of age in holoendemic *P. falciparum* transmission areas (Obonyo, *et al* 2007a) and, therefore, requires prompt recognition and treatment. In resource-poor settings, laboratory investigations are often limited, with diagnosis relying on medical history and physical examination (Chalco, *et al* 2005). However, the risk factors and clinical predictors of SMA have not been fully elucidated.

In sub-Saharan Africa, SMA may develop in children already debilitated by other causes of paediatric anaemia, such as nutritional deficiencies, haemoglobinopathies, bacteraemia, and human immunodeficiency virus-1 (HIV-1) (Berkley, *et al* 1999, Ong'echa, *et al* 2006, Otieno, *et al* 2006, Calis, *et al* 2008). These co-morbidities are common in African children and likely impact on the development and outcomes of malarial anaemia by altering the host immune response. We have recently shown that HIV-1 positive children have significantly more SMA during acute *P. falciparum* infections (Otieno, *et al* 2006). Moreover, bacterial pathogens, such as non-typhoid *Salmonella*, are common causes of bacteraemia in febrile, hospitalized children in sub-Saharan Africa and account for increased anaemia severity (Berkley, *et al* 1999, Graham, *et al* 2000, Walsh, *et al* 2000).

Since a number of previous studies contained an unknown prevalence of HIV-1 and bacteraemia (Luckner, *et al* 1998, Kahigwa, *et al* 2002, Verhoef, *et al* 2002, Ong'echa, *et al* 2006), it is difficult to interpret the impact of clinical, nutritional, demographic and socioeconomic factors on malarial anaemia severity. Although it is apparent that HIV-1 and bacteraemia can contribute to more profound anaemia in children with malaria, it is important to define SMA as a single disease entity so that gaps in our current knowledge about the patho-physiological basis of SMA can be advanced. As such, we determined the demographic, clinical and haematological parameters associated with SMA using a hospital-based cross-sectional study design in a holoendemic *P. falciparum* transmission area of western Kenya. Children with *P. falciparum* parasitaemia and varying severities of anaemia (3-36 mos. of age) were recruited at their first hospital contact for the treatment of febrile illness and all children found to have HIV-1 or bacteraemia, and those with CM or non-falciparum malaria, were excluded from the analyses.

PATIENTS, METHODS, AND MATERIALS

Study design and participants

Children were recruited from the paediatric ward of the Siaya District Hospital (SDH) in Siaya District, Nyanza Province, western Kenya, a holoendemic *P. falciparum* transmission area with an entomologic inoculation rate of 100-300 per annum (Beier, *et al* 1994). The prevalence of anaemia in Siaya District ranges between 60-90% in children less than five years of age (Bloland, *et al* 1999, McElroy, *et al* 2000). A detailed description of the study area, clinical facilities and study participant population is presented in our previous publication (Ong'echa, *et al* 2006).

Children of both genders (n=355, males=194 and females=161, aged 3-36 mos.) with fever and a positive blood film for *P. falciparum* (upon screening) were enrolled in the study from March, 2004 to January 2006. Children with a history of prior hospitalization (for any reason), those that received anti-malarial treatment within the previous two weeks, children with CM (an infrequent occurrence in western Kenya), and those with malaria from non-falciparum species were not eligible for participation. Since HIV-1 and bacteraemia are common co-pathogens that influence anaemia status in children with malaria (Berkley, *et al* 1999, Ong'echa, *et al* 2006, Otieno, *et al* 2006, Calis, *et al* 2008), all study participants were screened for HIV-1 and bacteraemia according to our previous methods (Ong'echa, *et al* 2006, Otieno, *et al* 2006, Ouma, *et al* 2008), and those found to be positive were excluded from the analyses. Pre- and post-test HIV counselling was provided to the parents/guardians of all participating children.

Based on haemoglobin (Hb) measurements, children with any density *P. falciparum* parasitaemia were placed into the following categories: uncomplicated malaria (Hb $\geq$ 11.0 g/dL); non-SMA (6.0 g/dL $\leq$ Hb<11.0 g/dL); and severe malarial anaemia (SMA, Hb<6.0 g/dL). Since precise definitions of childhood anaemia differ according to geographic regions, information from a previous study that performed >14,000 longitudinal Hb measurements in children (< 48 mos.) in western Kenya (McElroy, *et al* 1999) was used to appropriately define SMA as Hb<6.0 g/dL. To place the study into a broader context, data are also presented according to the World Health Organization (WHO) definition of SMA (Hb<5.0 g/dL with any density parasitaemia) (WHO 2000).

Treatment was prescribed according to Ministry of Health, Kenya (MOH) guidelines, which included Coartem® (an Artemether and Lumefantrine combination) for uncomplicated malaria, intravenous quinine for severe malaria, and supportive care (i.e., haematinics and blood transfusions). Human subject approval was obtained from the University of Pittsburgh, USA; University of New Mexico, USA; and KEMRI, Kenya.

Clinical assessment

History of present illness was obtained from the child's parent/guardian to assess findings indicative of malaria severity (Warrell, *et al* 1990, Marsh, *et al* 1995). Fever was defined as axillary temperature $>37.5^{\circ}\text{C}$, and duration of fever was recorded as none, 1-3 and 4-14 days. Hypotension, tachycardia and tachypnea were adjusted according to age (Marx 2002). Respiratory distress was defined as the presence of any of the following signs: alar flaring, chest retraction, use of accessory muscles during respiration, or abnormally deep, acidotic breathing (Marsh, *et al* 1995). Dehydration was defined as presence of sunken eyes or fontanelles, dry mucosa or decreased skin turgor. Nutritional status was determined by assessment of visible malnutrition, coded as "poor or fair" vs. "good" (Berkley, *et al* 2005a), while height-for-age (stunting), weight-for-height (wasting), weight-for-age (underweight), mid upper-arm circumference (MUAC)-for-height, MUAC-for-age and head circumference-

for-age Z-scores were determined according to our previous methods (Ong'echa, *et al* 2006). Markers of nutrition were therefore clinical rather than laboratory-based.

Laboratory investigations

Venous blood samples (<3.0 mL) were collected in EDTA-containing tubes at the time of enrolment, prior to initiation of supportive care or other treatment interventions. Presence and density of malaria parasites, and haematological measures, including full blood count, reticulocyte count and reticulocyte production index (RPI) were performed according to our previous studies (Were, *et al* 2006). The presence, number and density of malarial pigment (haemozoin)-containing monocytes (PCM) and neutrophils (PCN) were also determined per previous methods (Nguyen, *et al* 1995, Lyke, *et al* 2003, Awandare, *et al* 2007). To ensure accurate laboratory results, 8% of the samples were selected based on a random number generating algorithm and re-evaluated for all preceding measures. Peripheral blood smears (n=81) were also randomly selected (using a random number generator) from all groups for examination of RBC and WBC morphology, since some haematological characteristics are not detected with the automated methods. RBCs were examined for presence of sickle cells, leptocytes, schistocytes and spherocytes, while WBCs were assessed for lymphocytes and toxic granulations. Glucose levels were obtained with an Accu-check Compact® (Roche Diagnostics, Indianapolis, IN) glucometer. Haemoglobin variants and glucose-6-phosphate dehydrogenase (G6PD) deficiency were determined as described previously (Ouma, *et al* 2009). ABO phenotypes were determined by agglutination of RBCs with anti-A, anti-B, and anti-AB antisera (forward grouping).

Statistical analyses

Data were analyzed using SPSS (version 15.0, Chicago, Illinois, USA). Pearson's rank χ^2 test was used for comparing proportions. Group medians were compared using Kruskal–Wallis test followed by pairwise Mann–Whitney U test when significant Kruskal–Wallis tests were obtained. Logistic regression was used to examine the clinical predictors of SMA (statistical significance set at $P \leq 0.05$). All variables significantly associated with anaemia severity in the descriptive analyses, and in the comparisons among proportions, were divided into three clusters (laboratory parameters, patient history and physical examination). For each of these three separate clusters, a binomial logistic regression analysis was conducted. The purpose of these cluster-specific regressions was to reduce the overall number of predictors in the final predictor set and thereby reduce the probability of committing Type I errors. All variables from the cluster-specific regressions that were significant at $P \leq 0.05$ were then examined, along with age, as predictors of SMA in two additional second-step regression analyses. Non-SMA (alternatively coded as Hb=6.0-10.9 or 5.0-10.9 g/dL) and SMA (alternatively coded as Hb<6.0 or <5.0 g/dL) were used as categorical dependent variables. For both regression models, non-SMA was used as the comparison group since the uncomplicated malaria group was excluded from the regression analyses due to small sample sizes.

RESULTS

Demographic and laboratory parameters

Of the 671 subjects presenting at hospital with acute febrile illness and eligible for enrolment, children with bacteraemia (n=191), HIV-1 (n=24) and absence of *P. falciparum* parasitaemia (n=101) were excluded from the analyses. The remaining *P. falciparum*(+) children (3 to 36 mos., n=355) were stratified into three clinical categories: uncomplicated malaria (UM, Hb $\geq$ 11.0 g/dL, n=23); non-SMA (Hb 6.0-10.9 g/dL, n=200); and SMA (Hb<6.0 g/dL, n=132). Age and parasitaemia differed significantly across the groups, while none of the other parameters reached significance (Table I).

Haematological characteristics

Haematological characteristics of the study participants are shown in Table 1. Based on *a priori* grouping according to anaemia status, Hb, haematocrit and RBCs differed across the groups. MCV, MCH and MCHC were highest in UM, while RDW was highest in SMA. Although anaemic individuals (non-SMA and SMA) displayed reticulocytosis, the number of children with an RPI<2 [deficient erythroid proliferation/maturation (Hillman and Finch 1996)] was elevated in SMA (81%) vs. non-SMA (72%, $P=0.052$). Mild leukocytosis was present in the UM and SMA categories. Children with UM had ~two-fold higher neutrophil counts, while lymphocyte and monocyte counts increased with anaemia severity. Eosinophilia was rare (data not presented) and basophils were not recorded. Platelet counts progressively declined, while MPV increased with anaemia severity. Examination of randomly selected peripheral blood smears ($n=81$) revealed the following: leptocytes, schistocytes, acanthocytes and spherocytes were similar across the groups; macrocytosis was rare; and sickled cells were not detected. Nucleated RBC were observed in 4 of 17 (23.5%) SMA cases compared to 2 of 44 (4.5%) in the non-SMA group ($P=0.053$). Reactive lymphocytes were observed in ~15% of the children with equal distribution across the groups ($P=0.367$).

Genetic characteristics

Although heterozygous carriers of the HbS gene (HbAS) have 60-90% protection against acute malaria (Raper 1955), and reduced all-cause childhood mortality (Aidoo, *et al* 2002), HbAS was not significantly different (Table 1). Similarly, while G6PD deficiency confers a selective advantage against malaria (Bienzle, *et al* 1979), G6PD deficiency was also comparable (Table 1). Consistent with blood group O providing protection against severe malaria (Pathirana, *et al* 2005, Rowe, *et al* 2007), O⁺ individuals were over-represented in the UM, however, the across group difference was not significant (Table 1).

Haemozoin-containing neutrophils and monocytes

Since we recently demonstrated that elevated PCM was associated with increased risk of SMA (Awandare, *et al* 2007), presence of PCM and PCN were examined. PCN was substantially lower than PCM in all categories (Table 1). PCM prevalence and PCM/ μ L increased with anaemia severity, while PCN proportions and PCN/ μ L were similar across the groups (Table 1).

History of present illness and physical exam findings

Clinical characteristics of the study participants are shown in Table II. Though history of fever was frequent (>90%), recent febrile episodes (1-3 days) were more common in mild anaemia (78% UM, 59% non-SMA and 39% SMA, $P<0.001$), whereas prolonged fever (4-14 days) was coupled with enhanced anaemia (13% UM, 30% non-SMA and 54% SMA, $P<0.001$). Emesis was similar across groups, however, protracted emesis (4-14 days) was associated with more profound anaemia (9% UM, 14% non-SMA and 25% SMA, $P=0.025$). History of generalized pallor, respiratory distress, palmar and conjunctival pallor, splenomegaly and scleral icterus significantly differed and increased with anaemia severity. Visual nutritional status characterized as “fair or poor” upon physical examination was commonly observed and progressively increased with anaemia severity. Clinical assessment of height-for-age (*stunting*), weight-for-height (*wasting*), weight-for-age (*underweight*), head circumference-for-age, MUAC-for-age and MUAC-for-height Z scores (i.e., ≤ -2 , ≤ -3 or ≤ -4) revealed only one significant across group finding: a higher proportion of MUAC-for-height Z scores ≤ -2 in SMA (46%) vs. non-SMA (26%) and UM (23%) ($P=0.004$).

Predictors of SMA

To identify predictors of SMA (Hb<6.0 g/dL), logistic regression models were constructed. Cluster-specific regressions identified the following statistically significant variables: age; reticulocyte count; platelet count; prevalence of PCM; history of pallor; fever duration; conjunctival and palmar pallor; and splenomegaly (Table III). Among these variables, age, reticulocyte count, prevalence of PCM, history of pallor, and conjunctival and palmar pallor emerged as significant predictors of SMA in the second-step (final) regression analysis (Table III).

Stratification according to the WHO definition of SMA (Hb<5.0 g/dL) yielded 67 children in the SMA group and 265 in the non-SMA group. The initial cluster-specific regressions revealed that age, MCV, platelet count, prevalence of PCM, history of pallor, history of convulsions, palmar pallor and respiratory distress were significant predictors of SMA (Table III). In the second-step (final) regression analysis, MCV, prevalence of PCM, history of convulsions and palmar pallor were significant predictors of SMA (Table III). Respiratory distress was associated with a 4.8-fold higher risk of SMA, but results did not reach statistical significance ($P=0.068$, Table III).

DISCUSSION

Severe malarial anaemia is the most common clinical manifestation of severe malaria in infants and young children in holoendemic *P. falciparum* transmission areas and accounts for the greatest amount of global malaria-related morbidity and mortality (Bremner, *et al* 2001, WHO 2005). In a retrospective investigation of hospital admissions in Siaya District, the same hospital as the current study, severe anaemia accounted for 53% of the malaria-related deaths (Obonyo, *et al* 2007). Children in Siaya, as with many other holoendemic *P. falciparum* transmission areas, have a high rate of co-morbidities that can have a substantial impact on the development of SMA (Otieno, *et al* 2006). As such, it becomes difficult to discern the haematological and clinical factors characteristic of SMA versus those that arise due to other pathogens. It was therefore important to comprehensively examine clinical predictors and haematological characteristics of SMA in a large cohort of children in the absence of known co-morbidities that influence the course and outcomes of malarial anaemia.

The current study focused on SMA in infants and young children (3 to 36 mos.), a paediatric population highly susceptible to severe anaemia, as evidenced by a recent investigation in Siaya showing that SMA is found almost exclusively (i.e., 89% of the cases) in children less than three yrs. of age (Obonyo, *et al* 2007). This former study, one of few large cohorts investigated in a holoendemic *P. falciparum* transmission area, also illustrated that SMA has a 26% prevalence rate in children aged one to five years of age (Obonyo, *et al* 2007). As such, children selected for the present study were recruited during their first hospital contact for the treatment of paediatric illness so that they fell within this important age range. In addition, children were not eligible for enrolment if they had a prior hospitalization (for any reason) or transfusion. This strategy was aimed at reducing the potential confounding effects associated with previous illnesses, while at the same time, enriching the overall number of SMA cases presenting at hospital.

Consistent with our previous investigation in western Kenya (Ong'echa, *et al* 2006), anaemia severity was not associated with malaria parasite levels. For example, children with uncomplicated malaria had the highest peripheral parasitaemia, geomean parasitaemia and proportion of HDP, suggesting that parasite burden *per se* is not the primary factor responsible for the development of SMA. Although beyond the scope of the current manuscript, numerous investigations by our group support this finding and suggest that the

immune response to *P. falciparum*, rather than the cumulative parasite burden, is responsible for promoting SMA in holoendemic regions (Awandare, *et al* 2006, Ong'echa, *et al* 2006, Ouma, *et al* 2006).

As shown in Table 1, children with malarial anaemia (i.e., non-SMA and SMA) had a lower MCV and MCH than individuals with uncomplicated malaria. Moreover, MCV was a significant predictor of SMA (Hb<5.0 g/dL) in the multivariate model. Although it is possible that iron deficiency may contribute to the current findings, these parameters were not determined since it was difficult to obtain a large enough blood volume from small, anaemic children to perform a number of desired investigations. Increasing reticulocytosis and an elevated reticulocyte count were also significantly associated with enhanced anaemia severity. However, the prevalence of children with ineffective erythropoiesis (i.e., RPI<2) was also elevated in the non-SMA group (72%) and highest in children with SMA (81%), supporting our previous results demonstrating that malarial anaemia, particularly SMA, in holoendemic regions is characterized by ineffective erythropoiesis and/or bone marrow suppression (Keller, *et al* 2009, Were, *et al* 2009).

Thrombocytopaenia is a commonly observed haematological finding in malaria-infected individuals and typically presents in the mild-to-moderate range (Ladhani, *et al* 2002). During a malarial infection, the lifespan of platelets is reduced through a number of defined mechanisms, including platelet adhesion to the endothelial cells and RBCs, and increased platelet clearance in the setting of hypersplenism (Kueh and Yeo 1982). Findings here show that as anaemia progressively worsens, platelet counts decrease with accompanying splenomegaly. Concomitantly, these results suggest that hypersplenism may, at least in part, contribute to thrombocytopaenia in the cohort. Consistent with previous findings (Abdalla and Pasvol 2004), however, platelet count did not emerge as a significant predictor of anaemia severity.

Studies conducted by our group (Keller, *et al* 2009, Were, *et al* 2009, Were, *et al* 2006) and others (Akman-Anderson, *et al* 2007, Casals-Pascual, *et al* 2006, Prato, *et al* 2008) support the notion that acquisition of malarial pigment (haemozoin) by leukocytes promotes dysregulation in cytokines, chemokines and effector molecules that, in turn, are associated with suppression of erythropoiesis. Moreover, from a clinical standpoint, density of haemozoin in mononuclear cells (i.e., PCM) is a better correlate of disease severity than parasite density (Awandare, *et al* 2007b, Lyke, *et al* 2003, Metzger, *et al* 1995). Since the lifespan of monocytes is substantially longer than that of neutrophils (Khanna-Gupta and Berliner 2008, Whitelaw 1966), prevalence of PCM can provide insight into the duration of illness. In the present study, children with SMA had a higher prevalence and density of PCM than PCN, suggesting that SMA is characterized by a longer duration of illness, or alternatively, repeated episodes of untreated subclinical malarial infections. Prevalence of PCM was also one of the strongest predictors of risk for developing SMA (at both Hb<5.0 and <6.0 g/dL, respectively) demonstrating its importance as a marker of disease severity.

In addition to presence of PCM suggesting a more protracted illness in children with SMA, the longer duration of fever, emesis and poor feeding in this group further suggests that these children have been ill for an extended period of time prior to presentation at hospital compared to children in the UM and non-SMA groups. These data are consistent with findings in Colombia showing that paediatric and adult populations with malarial anaemia have an inverse relationship between Hb levels and duration of illness (Zamora, *et al* 2005). Results presented here showing that parasitaemia was not correlated with anaemia, and that scleral icterus was only rarely observed, also parallel those investigations (Zamora, *et al* 2005). Thus, intravascular haemolysis caused by hyperparasitaemia does not likely explain the development of anaemia in the current cohort.

It is important to note that although children with SMA had a more prolonged duration of fever, the prevalence of axillary temperature ($>37.5^{\circ}\text{C}$) was actually lower in children with SMA upon presentation at hospital. These data support findings in Burkina Faso in which afebrile *P. falciparum* parasitaemia contributed to a high prevalence of moderate to severe anaemia in children 6 to 23 mos. of age (Ouedraogo, *et al* 2008).

Anaemia may be difficult to recognize in resource-poor settings where basic laboratory investigations are not always available. Pallor has been extensively evaluated as a clinical sign of anaemia in infants and young children in malaria endemic areas (Chalco, *et al* 2005, Obonyo, *et al* 2006). Findings in the current study illustrate that the parents/guardians were readily able to identify pallor. History of pallor, along with the identification of pallor (conjunctival and palmar) by clinicians were significantly associated with enhanced anaemia severity ($\text{Hb}<6.0\text{ g/dL}$), while only palmar pallor was a significant predictor of SMA when using the WHO criteria of SMA ($\text{Hb}<5.0\text{ g/dL}$). Identification of pallor by caregivers and clinical staff therefore represents an important clinical sign that can be used to identify more profound anaemia.

Respiratory distress is a strong correlate of life-threatening malaria in non-holoendemic transmission areas (Marsh, *et al* 1995). In the current study, respiratory distress was strongly associated with SMA in the univariate analyses, although it was not significantly associated with SMA in the final regression models using either definition of SMA (i.e., $\text{Hb}<5.0$ or $<6.0\text{ g/dL}$). However, the nearly 5-fold increased risk of developing SMA associated with respiratory distress when using the WHO criteria of SMA demonstrates that respiratory distress is highly important and may not have reached statistical significance in our cohort due to sample size issues. The finding that tachypnea was not significantly correlated with SMA appears to suggest that the patho-physiological process underlying respiratory distress was related to metabolic acidosis, rather than shallow rapid breathing due to pulmonary edema. This interpretation is consistent with investigations showing a high prevalence of metabolic acidosis in children with severe malaria in which respiratory rate and auscultatory findings failed to predict disease severity (Marsh, *et al* 1995).

Convulsions emerged as a significant predictor of SMA in the final regression model, but only when using the WHO definition of SMA [i.e., $\text{Hb}<5.0\text{ g/dL}$ (Snow, *et al* 1999a)]. Although a history of convulsions was reported by the childrens' parents/guardians, convulsions were not observed by clinicians when children presented at hospital, nor were convulsions significantly correlated with fever, age or hypoglycaemia (data not presented), suggesting that children may have had benign febrile seizures prior to presentation at hospital that did not manifest as neurological sequelae upon physical examination.

Micro- and macro-nutrient deficiencies are common in sub-Saharan Africa and likely impair the host immune response to parasitic diseases, possibly preventing the development of semi-immunity to severe malaria that occurs in endemic transmission areas. However, previous studies have shown that malnourished children are partially protected against malaria (McGregor 1982). This counterintuitive finding underscores the complexity of the interaction between the host immune system and the malaria parasite, and may be explained by postulating that a robust parasite-driven, pro-inflammatory state elicited in a well-nourished, fully immune-competent host could be maladaptive, and paradoxically, lead to higher malaria-related morbidity. In our study, we were interested in exploring the effect of clinical nutritional markers on malarial anaemia. Although the univariate analyses showed a trend towards a higher prevalence of markers of malnutrition in the SMA group, nutritional parameters did not emerge as significant predictors of SMA in the final regression model, a finding that confirms previous observations by our group in this region (Ong'echa, *et al* 2006). One explanation for this finding is that the effect of iron deficiency outweighs that of

other nutritional deficiencies and adversely affects erythropoiesis. Thus, iron deficiency may mediate the correlation observed between malnutrition and malarial anaemia. Although we did not directly test for iron deficiency, a limitation imposed by the need to minimize the risk of iatrogenic anaemia, the significant correlation between a low MCV and SMA in the final regression model supports previous reports where stunted patients with malaria were more likely to have severe anaemia and a high serum transferrin receptor concentration, a sensitive marker of iron deficiency (Verhoef, *et al* 2002). However, this postulate needs to be validated since a recent study in preschool children in Malawi showed that iron deficiency was highest in community and hospital control patients relative to those with SMA (Calis, *et al* 2008). A previous study in western Kenya, conducted in children in the same age range, showed that stunting was more common in children with SMA (Friedman, *et al* 2005). This apparent discrepancy with findings presented here may be related to the fact that the confounding effects of HIV-related malnutrition were excluded in our population.

A previous study exploring the risk factors associated with malarial anaemia in Zambian children aged 6 to 119 mos. found that hookworm infestation was present in only 1.2% of the study participants (Mulenga, *et al* 2005). Since the highest prevalence of hookworm in African children typically occurs between six to ten years of age (Diemert, *et al* 2008), an even lower prevalence of hookworm infections may be expected in children less than 36 mos. of age examined here. Comprehensive hookworm infestation data were not available in the current study due to difficulty in obtaining stool samples, particularly in very ill children. As such, the influence of hookworm infections on anaemia outcomes presented here cannot be definitively ruled out.

In summary, we have comprehensively characterized the demographic, clinical, and haematological parameters associated with SMA and identified a number of predictors of SMA in pre-school children from western Kenya. Significant predictors of SMA when using a definition of Hb<6.0 g/dL, include: age; reticulocyte count; PCM; history of pallor and conjunctival and palmar pallor, whereas significant predictors of SMA when using a definition of Hb<5.0 g/dL, include: MCV; PCM; history of convulsions; and palmar pallor. Thus, it appears that there are both unique and overlapping clinical predictors of SMA depending on the definition of anaemia severity employed. Nevertheless, it is clear that both pallor and intra-monocytic hemozoin deposition can be used to identify those children that suffer from SMA. Results presented here have direct clinical implications since identification of the clinical predictors discovered in the current study may aid in the prompt recognition of children that require rapid and appropriate therapeutic management.

Acknowledgments

The authors are grateful to all of the study participants and their families, and the University of New Mexico-KEMRI staff. We also thank the Siaya District Hospital staff and management, and the cooperation of the Director of Kenya Medical Research Institute (KEMRI) for approving this manuscript for publication.

REFERENCES

- Abdalla, S.; Pasvol, G. *Malaria: A Haematological Perspective*. Imperial College Press; London: 2004.
- Aidoo M, Terlouw DJ, Kolczak MS, McElroy PD, ter Kuile FO, Kariuki S, Nahlen BL, Lal AA, Udhayakumar V. Protective effects of the sickle cell gene against malaria morbidity and mortality. *Lancet*. 2002; 359:1311–1312. [PubMed: 11965279]
- Akman-Anderson L, Olivier M, Luckhart S. Induction of nitric oxide synthase and activation of signaling proteins in *Anopheles* mosquitoes by the malaria pigment, hemozoin. *Infection and Immunity*. 2007; 75:4012–4019. [PubMed: 17526741]

- Awandare GA, Ouma C, Keller CC, Were T, Otieno R, Ouma Y, Davenport GC, Hittner JB, Ong'echa JM, Ferrell R, Perkins DJ. A macrophage migration inhibitory factor promoter polymorphism is associated with high-density parasitaemia in children with malaria. *Genes and Immunity*. 2006; 7:568–575. [PubMed: 16929348]
- Awandare GA, Ouma Y, Ouma C, Were T, Otieno R, Keller CC, Davenport GC, Hittner JB, Vulule J, Ferrell R, Ong'echa JM, Perkins DJ. Role of monocyte-acquired haemozoin in suppression of macrophage migration inhibitory factor in children with severe malarial anaemia. *Infection and Immunity*. 2007; 75:201–210. [PubMed: 17060471]
- Beier JC, Oster CN, Onyango FK, Bales JD, Sherwood JA, Perkins PV, Chumo DK, Koech DV, Whitmire RE, Roberts CR, et al. *Plasmodium falciparum* incidence relative to entomologic inoculation rates at a site proposed for testing malaria vaccines in western Kenya. *American Journal of Tropical Medicine and Hygiene*. 1994; 50:529–536. [PubMed: 7911282]
- Berkley J, Mwangi I, Griffiths K, Ahmed I, Mithwani S, English M, Newton C, Maitland K. Assessment of severe malnutrition among hospitalized children in rural Kenya: comparison of weight for height and mid upper arm circumference.[see comment]. *Journal of the American Medical Association*. 2005a; 294:591–597. [PubMed: 16077053]
- Berkley J, Mwarumba S, Bramham K, Lowe B, Marsh K. Bacteraemia complicating severe malaria in children. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 1999; 93:283–286. [PubMed: 10492760]
- Berkley JA, Lowe BS, Mwangi I, Williams T, Bauni E, Mwarumba S, Ngetsa C, Slack MP, Njenga S, Hart CA, Maitland K, English M, Marsh K, Scott JA. Bacteraemia among children admitted to a rural hospital in Kenya. *New England Journal of Medicine*. 2005b; 352:39–47. [PubMed: 15635111]
- Bienze U, Guggenmoos-Holzmänn I, Luzzatto L. Malaria and erythrocyte glucose-6-phosphate dehydrogenase variants in West Africa. *American Journal of Tropical Medicine and Hygiene*. 1979; 28:619–621. [PubMed: 464185]
- Boland PB, Boriga DA, Ruebush TK, McCormick JB, Roberts JM, Oloo AJ, Hawley W, Lal A, Nahlen B, Campbell CC. Longitudinal cohort study of the epidemiology of malaria infections in an area of intense malaria transmission II. Descriptive epidemiology of malaria infection and disease among children. *American Journal of Tropical Medicine and Hygiene*. 1999; 60:641–648. [PubMed: 10348241]
- Bremner JG, Egan A, Keusch GT. The intolerable burden of malaria: a new look at the numbers. *American Journal of Tropical Medicine and Hygiene*. 2001; 64:iv–vii. [PubMed: 11425185]
- Calis JC, Phiri KS, Faragher EB, Brabin BJ, Bates I, Cuevas LE, de Haan RJ, Phiri AI, Malange P, Khoka M, Hulshof PJ, van Lieshout L, Beld MG, Teo YY, Rockett KA, Richardson A, Kwiatkowski DP, Molyneux ME, van Hensbroek MB. Severe anaemia in Malawian children. *New England Journal of Medicine*. 2008; 358:888–899. [PubMed: 18305266]
- Casals-Pascual C, Kai O, Cheung JO, Williams S, Lowe B, Nyanoti M, Williams TN, Maitland K, Molyneux M, Newton CR, Peshu N, Watt SM, Roberts DJ. Suppression of erythropoiesis in malarial anaemia is associated with haemozoin *in vitro* and *in vivo*. *Blood*. 2006; 108:2569–2577. [PubMed: 16804108]
- Chalco JP, Huicho L, Alamo C, Carreazo NY, Bada CA. Accuracy of clinical pallor in the diagnosis of anaemia in children: a meta-analysis. *BMC Pediatrics*. 2005; 5:46. [PubMed: 16336667]
- Diemert DJ, Bethony JM, Hotez PJ. Hookworm vaccines. *Clinical Infectious Diseases*. 2008; 46:282–288. [PubMed: 18171264]
- Dzeing-Ella A, Nze Obiang PC, Tchoua R, Planche T, Mboza B, Mbounja M, Muller-Roemer U, Jarvis J, Kendjo E, Ngou-Milama E, Kremsner PG, Krishna S, Kombila M. Severe falciparum malaria in Gabonese children: clinical and laboratory features. *Malaria Journal*. 2005; 4:1. [PubMed: 15638948]
- English M, Wale S, Binns G, Mwangi I, Sauerwein H, Marsh K. Hypoglycaemia on and after admission in Kenyan children with severe malaria. *Quarterly Journal of Medicine*. 1998; 91:191–197.
- Friedman JF, Kweni AM, Mirel LB, Kariuki SK, Terlouw DJ, Phillips-Howard PA, Hawley WA, Nahlen BL, Shi YP, ter Kuile FO. Malaria and nutritional status among pre-school children:

- results from cross-sectional surveys in western Kenya. *American Journal of Tropical Medicine and Hygiene*. 2005; 73:698–704. [PubMed: 16222012]
- Graham SM, Walsh AL, Molyneux EM, Phiri AJ, Molyneux ME. Clinical presentation of non-typhoidal *Salmonella* bacteraemia in Malawian children. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 2000; 94:310–314. [PubMed: 10975008]
- Hillman, RS.; Finch, CA. *Red Cell Manual*. F.A. Davis; Philadelphia, PA: 1996.
- Keller CC, Ouma C, Ouma Y, Awandare GA, Davenport GC, Were T, Hittner JB, Vulule JM, Ong'echa JM, Perkins DJ. Suppression of a novel hematopoietic mediator in children with severe malarial anaemia. *Infection and Immunity*. 2009; 77:3864–3871. [PubMed: 19528216]
- Khanna-Gupta, A.; Berliner, N. Granulocytopoiesis and monocytopenia. In: Hoffman, R.; Benz, EJ.; Shattil, SJ.; Furie, B.; Silberstein, LE.; McGlave, P.; Heslop, H., editors. *Hematology: Basic Principles and Practice*. Churchill Livingstone: 2008.
- Kueh YK, Yeo KL. Haematological alterations in acute malaria. *Scandinavian Journal of Haematology*. 1982; 29:147–152. [PubMed: 6753122]
- Ladhani S, Lowe B, Cole AO, Kowuondo K, Newton CR. Changes in white blood cells and platelets in children with falciparum malaria: relationship to disease outcome. *British Journal of Haematology*. 2002; 119:839–847. [PubMed: 12437669]
- Lyke KE, Diallo DA, Dicko A, Kone A, Coulibaly D, Guindo A, Cissoko Y, Sangare L, Coulibaly S, Dakouo B, Taylor TE, Doumbo OK, Plowe CV. Association of intraerythrocytic *Plasmodium falciparum* malaria pigment with disease severity, clinical manifestations, and prognosis in severe malaria. *American Journal of Tropical Medicine and Hygiene*. 2003; 69:253–259. [PubMed: 14628940]
- Marsh K, Forster D, Waruiru C, Mwangi I, Winstanley M, Marsh V, Newton C, Winstanley P, Warn P, Peshu N, et al. Indicators of life-threatening malaria in African children.[see comment]. *New England Journal of Medicine*. 1995; 332:1399–1404. [PubMed: 7723795]
- Marx J. *Rosen's Emergency Medicine: Concepts and Clinical Practice*. 2002
- McElroy PD, ter Kuile FO, Lal AA, Bloland PB, Hawley WA, Oloo AJ, Monto AS, Meshnick SR, Nahlen BL. Effect of *Plasmodium falciparum* parasitaemia density on haemoglobin concentrations among full-term, normal birth weight children in western Kenya, IV. The Asembo Bay Cohort Project. *American Journal of Tropical Medicine and Hygiene*. 2000; 62:504–512. [PubMed: 11220768]
- McGregor IA. Malaria: nutritional implications. *Reviews in Infectious Disease*. 1982; 4:798–804.
- Metzger WG, Mordmuller BG, Kremsner PG. Malaria pigment in leucocytes. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 1995; 89:637–638. [PubMed: 8594680]
- Mockenhaupt FP, Ehrhardt S, Burkhardt J, Bosomtwe SY, Laryea S, Anemana SD, Otchwemah RN, Cramer JP, Dietz E, Gellert S, Bienzle U. Manifestation and outcome of severe malaria in children in northern Ghana. *American Journal of Tropical Medicine and Hygiene*. 2004; 71:167–172. [PubMed: 15306705]
- Mulenga M, Malunga P, Bennett S, Thuma PE, Shulman C, Fielding K, Greenwood BM. Factors associated with severe anaemia in Zambian children admitted with *Plasmodium falciparum* malarial anaemia. *Annals of Tropical Paediatrics*. 2005; 25:87–90. [PubMed: 15949196]
- Nguyen PH, Day N, Pram TD, Ferguson DJ, White NJ. Intraerythrocytic malaria pigment and prognosis in severe malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 1995; 89:200–204. [PubMed: 7778149]
- Obonyo, C.; Taylor, W.; Ogutu, B.; Vulule, J.; Grobbee, D. Prevalence of and risk factors for malaria-associated anaemia in young Kenyan children. University of Utrecht; Utrecht: 2006.
- Obonyo CO, Vulule J, Akhwale WS, Grobbee DE. In-hospital morbidity and mortality due to severe malarial anaemia in western Kenya. *American Journal of Tropical Medicine and Hygiene*. 2007; 77:23–28. [PubMed: 18165471]
- Ong'echa JM, Keller CC, Were T, Ouma C, Otieno RO, Landis-Lewis Z, Ochiel D, Slingluff JL, Mogere S, Ogonji GA, Orago AS, Vulule JM, Kaplan SS, Day RD, Perkins DJ. Parasitaemia, anaemia, and malarial anaemia in infants and young children in a rural holoendemic *Plasmodium falciparum* transmission area. *American Journal of Tropical Medicine and Hygiene*. 2006; 74:376–385. [PubMed: 16525094]

- Otieno RO, Ouma C, Ong'echa JM, Keller CC, Were T, Waindi EN, Michaels MG, Day RD, Vulule JM, Perkins DJ. Increased severe anaemia in HIV-1-exposed and HIV-1-positive infants and children during acute malaria. *AIDS*. 2006; 20:275–280. [PubMed: 16511422]
- Ouedraogo HZ, Zeba A, Dramaix-Wilmet M, Donnen P. Moderate-to-severe anaemia due to afebrile *Plasmodium falciparum* infection in children aged 6–23 months from the rural district of Kongoussi, Burkina Faso. *Journal of Tropical Paediatrics*. 2008
- Ouma C, Davenport GC, Were T, Otieno MF, Hittner JB, Vulule JM, Martinson J, Ong'echa JM, Ferrell RE, Perkins DJ. Haplotypes of IL-10 promoter variants are associated with susceptibility to severe malarial anaemia and functional changes in IL-10 production. *Human Genetics*. 2008; 124:515–524. [PubMed: 18972133]
- Ouma C, Keller CC, Davenport GC, Were T, Konah S, Otieno MF, Hittner JB, Vulule JM, Martinson J, Ong'echa JM, Ferrell RE, Perkins DJ. A novel functional variant in the Stem Cell Growth Factor (SCGF) promoter (–539C/T) protects against severe malarial anaemia. *Infection and Immunity*. 2009
- Ouma C, Keller CC, Opondo DA, Were T, Otieno RO, Otieno MF, Orago AS, Ong'Echa JM, Vulule JM, Ferrell RE, Perkins DJ. Association of FCgamma receptor IIA (CD32) polymorphism with malarial anemia and high-density parasitemia in infants and young children. *American Journal of Tropical Medicine and Hygiene*. 2006; 74:573–577. [PubMed: 16606986]
- Pathirana SL, Alles HK, Bandara S, Phone-Kyaw M, Perera MK, Wickremasinghe AR, Mendis KN, Handunnetti SM. ABO-blood-group types and protection against severe, *Plasmodium falciparum* malaria. *Annals of Tropical Medicine and Parasitology*. 2005; 99:119–124. [PubMed: 15814030]
- Prato M, Gallo V, Giribaldi G, Arese P. Phagocytosis of haemozoin (malarial pigment) enhances metalloproteinase-9 activity in human adherent monocytes: role of IL-1beta and 15-HETE. *Malaria Journal*. 2008; 7:157. [PubMed: 18710562]
- Raper AB. Malaria and the sickling trait. *British Medical Journal*. 1955; 1:1186–1189. [PubMed: 14363830]
- Rowe JA, Handel IG, Thera MA, Deans A-M, Lyke KE, Kone A, Diallo DA, Raza A, Kai O, Marsh K, Plowe CV, Doumbo OK, Moulds JM. Blood group O protects against severe *Plasmodium falciparum* malaria through the mechanism of reduced rosetting. *Proceedings of the National Academy of Sciences*. 2007; 104:17471–17476.
- Snow RW, Craig M, Deichmann U, Marsh K. Estimating mortality, morbidity and disability due to malaria among Africa's non-pregnant population. *Bulletin of the World Health Organization*. 1999a; 77:624–640. [PubMed: 10516785]
- Snow RW, Craig MH, Deichmann U, le Sueur D. A preliminary continental risk map for malaria mortality among African children. *Parasitol Today*. 1999b; 15:99–104. [PubMed: 10322322]
- Verhoef H, West CE, Veenemans J, Beguin Y, Kok FJ. Stunting may determine the severity of malaria-associated anaemia in African children. *Paediatrics*. 2002; 110:e48.
- Walsh AL, Phiri AJ, Graham SM, Molyneux EM, Molyneux ME. Bacteraemia in febrile Malawian children: clinical and microbiologic features. *Paediatric Infectious Disease Journal*. 2000; 19:312–318.
- Warrell D, Molyneux M, Beales P. Severe and complicated malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 1990; 84:1–65.
- Were T, Davenport GC, Yamo EO, Hittner JB, Awandare GA, Otieno MF, Ouma C, Orago AS, Vulule JM, Ong'echa JM, Perkins DJ. Naturally acquired haemozoin by monocytes promotes suppression of RANTES in children with malarial anaemia through an IL-10-dependent mechanism. *Microbes and Infection*. 2009
- Were T, Hittner JB, Ouma C, Otieno RO, Orago ASS, Ong'echa JM, Vulule JM, Keller CC, Perkins DJ. Suppression of RANTES in children with *Plasmodium falciparum* malaria.[erratum appears in *Haematologica*. 2007 Jan;92(1):136 Note: Hittner, J B [added]]. *Haematologica*. 2006; 91:1396–1399. [PubMed: 17018392]
- Whitelaw DM. The intravascular lifespan of monocytes. *Blood*. 1966; 28:455–464. [PubMed: 5918472]
- WHO. Severe falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 2000; 94(Supplement 1):S1–90. [PubMed: 11103309]

- WHO. WHO. World Malaria Report 2005. World Health Organization/United Nations Children's Fund; Geneva: 2005. 2005: http://www.rollbackmalaria.org/wmr2005/pdf/WMReport_lr.pdf
- Zamora F, Ramirez O, Vergara J, Arevalo-Herrera M, Herrera S. Haemoglobin levels related to days of illness, race, and *Plasmodium* species in Colombian patients with uncomplicated malaria. American Journal of Tropical Medicine and Hygiene. 2005; 73:50–54. [PubMed: 16291767]

Table I

Demographic, laboratory, haematological and genetic characteristics

	UM	Non-SMA	SMA	P	n
Demographic and Laboratory Indices					
Age (mos.)	9.0 (9.0)	10.5 (11.0)	8.0 (7.0)	0.002	355
Female gender, no. (%)	11 (48)	86 (43)	64 (48)	0.599	355
Peripheral parasitaemia (µL)	55,800 (115,055)	26,956 (55,984)	32,384 (66,997)	0.026	355
Geomean parasitaemia (µL)	48,675	21,595	22,709		355
HDP ^a no. (%)	20 (87)	132 (66)	83 (63)	0.079	355
Hypoglycaemia ^b no. (%)	2 (13)	21 (20)	19 (17)	0.768	230
Erythrocytic Indices					
Haemoglobin (g/dL)	11.5 (0.8)	7.8 (2.2)	4.9 (1.1)	<0.001	354
Haematocrit (%)	34.3 (3.0)	24.2 (5.5)	15.9 (4.2)	<0.001	354
RBC (x106/ml)	4.8 (0.9)	3.6 (1.1)	2.2 (0.8)	<0.001	354
MCV (fL)	75.6 (9.5)	68.8 (10.2)	70.4 (13.3)	0.009	354
MCH (pg)	24.8 (3.3)	22.2 (3.4)	22.3 (4.0)	<0.001	354
MCHC (g/dL)	33.4 (1.8)	32.2 (2.2)	31.9 (2.7)	<0.001	354
RDW (%)	17.0 (4.5)	20.7 (4.5)	23.1 (5.3)	<0.001	354
Reticulocyte count (%)	1.1 (1.8)	2.6 (3.6)	3.7 (5.9)	<0.001	354
RPI $<2^c$	NA	144 (72)	105 (81)	0.052	329
Leukocytic Indices					
WBC ($\times 10^9/L$)	13.5 (8.1)	10.3 (4.8)	13.0 (7.7)	0.001	353
Granulocytes ($\times 10^9/L$)	8.1 (6.5)	4.3 (3.3)	4.4 (4.0)	0.001	353
Lymphocytes ($\times 10^9/L$)	4.2 (2.1)	5.3 (3.2)	6.5 (5.1)	<0.001	353
Monocytes ($\times 10^9/L$)	0.7 (0.8)	0.9 (0.6)	1.3 (1.2)	<0.001	353
Platelet Indices					
Platelets ($\times 10^9/L$)	247 (168)	159 (133)	144 (81)	0.001	354

	UM	Non-SMA	SMA	P	n
MPV (fL)	7.4 (1.0)	8.3 (1.7)	8.6 (1.7)	<0.001	351
Genetic Characteristics					
Sickle-cell trait ^d , no. (%)	3 (13)	37 (18)	14 (11)	0.140	355
G6PD deficiency, no. (%)	0 (0)	11 (6)	9 (7)	0.422	333
Blood group O, no. (%)	12 (52)	87 (43)	52 (40)	0.502	354
Haemozoin-containing Leucocytes					
PCM, no. (%)	2 (9)	65 (33)	92 (70)	<0.001	355
PCN, no. (%)	2 (9)	11 (6)	13 (10)	0.320	355
PCM ^e (μL)	0.4 (0.2)	0.4 (1.6)	0.5 (3.1)	0.072	159
PCN (μL)	0.1 (0.1)	0.1 (0.9)	0.2 (0.4)	0.489	26

Data are presented as the median (interquartile range) unless otherwise noted. Differences between the groups were compared using Kruskal-Wallis test for medians, while proportions were compared using Pearson's χ^2 test.

^aHigh density parasitaemia defined as $\geq 10,000$ parasites/ μ L.

^bBlood glucose <4.2 mmol per liter (75 mg/dL).

^cThe difference between the non-SMA and SMA groups (only) was compared, since an RPI <2 is not valid in the non-anaemic uncomplicated malaria group.

^dPresence of haemoglobin AS by electrophoresis. Difference between SMA and Non-SMA groups was significant ($P = 0.025$, Mann-Whitney U)

Table II

Clinical characteristics

	UM	Non-SMA	SMA	P	n
Fever	21 (91)	185 (92)	128 (97)	0.202	355
Emesis	9 (39)	118 (58)	82 (62)	0.118	355
Poor feeding	16 (70)	144 (72)	104 (80)	0.247	355
Oedema	0 (0)	1 (1)	1 (1)	0.888	355
Jaundice	0 (0)	3 (1)	4 (3)	0.482	355
Patient History					
Difficulty breathing	2 (9)	14 (7)	12 (9)	0.779	355
Cough	6 (26)	72 (36)	43 (33)	0.573	355
Diarrhoea	8 (35)	86 (43)	52 (39)	0.658	355
Generalized pallor	1 (4)	27 (13)	48 (36)	<0.001	355
Convulsions	0 (0)	4 (2)	6 (4)	0.267	355
Unresponsiveness	0 (0)	2 (1)	0 (0)	0.463	355
Axillary temperature (°C) ^a	37.6 (2.5)	37.5 (1.9)	37.3 (1.4)	0.397	355
Axillary temperature >37.5°C	13 (56)	103 (51)	58 (44)	0.335	355
Heart rate ^a	124 (17)	124 (20)	131 (16)	0.362	350
Tachycardia ^b	2 (9)	13 (7)	7 (5)	0.793	350
Hypotension ^b	3 (33)	10 (18)	24 (37)	0.065	128
Respiratory rate ^a	32 (14)	32 (13)	32 (12)	0.755	337
Tachypnea ^b	3 (14)	17 (9)	13 (10)	0.740	336
Physical Exam					
Respiratory distress ^c	0 (0)	1 (1)	9 (7)	0.002	355
Palmar pallor	0 (0)	40 (20)	82 (63)	<0.001	354
Conjunctival pallor	1 (4)	84 (42)	101 (76)	<0.001	355
Dehydration ^d	0 (0)	8 (4)	8 (6)	0.372	355
Splenomegaly	0 (0)	27 (13)	40 (30)	<0.001	354
Hepatomegaly	0 (0)	2 (1)	1 (1)	0.877	354
Lymphadenopathy	0 (0)	10 (5)	7 (5)	0.532	354
Scleral icterus	0 (0)	0 (0)	4 (3)	0.032	355

	UM	Non-SMA	SMA	P	n
Fair or poor nutritional status ^e	11 (48)	108 (54)	100 (76)	<0.001	355

^aMedian (interquartile range) of axillary temperature, heart rate, and respiratory rate is reported. For all other clinical signs or symptoms, the number (%) of patients is reported.

^bTachycardia, hypotension, and tachypnea were age-adjusted according to standard reference values.

^cRespiratory distress was defined as the presence of any of the following signs: alar flaring; chestretraction; use of accessory muscles during respiration; or abnormally deep, acidotic breathing.

^dDehydration was defined as presence of sunken eyes or fontanelles, dry mucosa, or decreasedskin turgor.

^eNutritional status of the children was determined by assessment of visible malnutrition, coded as "good" vs. "fair or poor" .

Table III

Binomial logistic regression analyses examining clinical predictors of SMA

	Predictor	SMA (Hb<6.0 g/dL)			Predictor	SMA (Hb<5.0 g/dL)		
		OR	95%CI	P		OR	95%CI	P
Demographic	Age	0.94	0.90-0.98	0.006	Age	0.96	0.92-1.01	0.093
Laboratory	Reticulocyte count	1.08	1.01-1.17	0.026	MCV	1.04	1.01-1.07	0.009
	Platelet count	0.99	0.99-1.00	0.201	Platelet count	0.99	0.73-1.54	0.492
	PCM	4.37	2.39-7.97	<0.001	PCM	2.71	1.41-5.21	0.003
History	Pallor (history)	2.26	1.16-4.41	0.017	Pallor (history)	1.38	0.70-2.73	0.357
					Convulsions (history)	6.53	1.37-31.24	0.019
Physical Exam	Conjunctival pallor	2.16	1.10-4.22	0.025	Palmar pallor	3.71	1.97-6.97	<0.001
	Palmar pallor	4.24	2.21-8.11	<0.001	Respiratory distress	4.82	0.89-31.24	0.068
	Splenomegaly	1.88	0.92-3.83	0.081				

Final regression analyses performed using the two alternative Hb cut-off values to define SMA. All variables that emerged as significant predictors of SMA in the first-step regression analyses are shown within their respective regression clusters.

Odds Ratio (OR) and 95% confidence interval (CI).

Pigment-containing monocytes (PCM).

Mean corpuscular volume (MCV).