Inter-Relationships of Cardinal Features and Outcomes of Symptomatic Pediatric Plasmodium falciparum Malaria in 1,933 Children in Kampala, Uganda

Am. J. Trop. Med. Hyg., 88(4), 2013, pp. 747–756
doi:10.4269/ajtmh.12-0668

Inter-Relationships of Cardinal Features and Outcomes of Symptomatic Pediatric *Plasmodium falciparum* Malaria in 1,933 Children in Kampala, Uganda

Christine M. Cserti-Gazdewich, Aggrey Dhabangi, Charles Musoke, Isaac Ssewanyana, Henry Ddungu, Deborah Nakiboneka-Ssenabulya, Nicolette Nabukeera-Barungi, Arthur Mpimbaza, and Walter H. Dzik*

University Health Network, University of Toronto, Toronto, Canada; Mulago Hospital, Makerere University College of Health Sciences, Kampala, Uganda; Joint Clinical Research Center, Kampala, Uganda; Uganda Cancer Institute and the African Palliative Care Association, Kampala, Uganda; Massachusetts General Hospital, Harvard University, Boston, Massachusetts

Abstract. Malaria remains a challenging diagnosis with variable clinical presentation and a wide spectrum of disease severity. Using a structured case report form, we prospectively assessed 1,933 children at Mulago Hospital in Kampala, Uganda with acute *Plasmodium falciparum* malaria. Children with uncomplicated malaria significantly differed from those with severe disease for 17 features. Among 855 children with severe disease, the case-fatality rate increased as the number of severity features increased. Logistic regression identified five factors independently associated with death: cerebral malaria, hypoxia, severe thrombocytopenia, leukocytosis, and lactic acidosis. Cluster analysis identified two groups: one combining anemia, splenomegaly, and leukocytosis; and a second group centered on death, severe thrombocytopenia, and lactic acidosis, which included cerebral malaria, hypoxia, hypoglycemia, and hyper-parasitemia. Our report updates previous clinical descriptions of severe malaria, quantifies significant clinical and laboratory inter-relationships, and will assist clinicians treating malaria and those planning or assessing future research (NCT00707200) (www.clinicaltrials.gov).

INTRODUCTION

Children with malaria have a wide variety of signs and symptoms. The World Health Organization recognizes numerous hallmark features of severe malaria (Table 1).[1] Acute malaria syndromes carry diagnostic value and prognostic importance, but do not occur with equal prevalence among different age groups and across different regions. The severity of clinical infection in malaria depends on complex interactions of host, parasite, and environmental factors.[1]

Numerous previous studies have analyzed clinical features in malaria. Early work by Marsh and others established that three overlapping syndromes were found in severe disease: cerebral malaria (CM), respiratory distress (RD), and severe malaria anemia (SMA).[2,3] Subsequent reports further characterized the clinical findings of malaria and established fundamental patterns of the illness.[4–14] These patterns include the importance of impaired consciousness as a risk-factor for fatal outcome, the value of blood transfusion in the treatment of severe anemia, hypoglycemia during acute illness, and the observation of RD as a manifestation of lactic acidosis (LA). These studies were published more than 15 years ago and most were based on < 500 patients.

During 2000–2010, additional reports refined the case definitions of severe malaria. Two studies of children with severe malaria in Gabon and a third study from Mali reported that mortality was associated with CM, hypoglycemia, RD and LA.[15–17] These reports were unable to assess the contribution of increased blood lactate levels, thrombocytopenia, and leukocytosis to outcomes. Idro and others[18] provided a detailed description of 100 children with CM treated in Uganda and identified RD, circulatory failure, hyporeflexia, and hyperparasitemia as additive risk factors for fatal outcomes. In a subsequent report of more than 9,000 children in Kenya with malaria, they confirmed that acidosis, hypoglycemia, and circulatory collapse were associated with neurologic signs.[19] Smaller studies from Ghana[20] and Gabon[21] and a multicenter sub-Saharan study[22] further defined complications of severe disease. In a review of 25 previously published studies, Roca-Feltrer and others reported that the age distribution for SMA was consistently younger than that for CM.[23] Recently, Vekemans and others[24] provided a thorough review of the published literature through 2010 and suggested a standardized case definition for severe malaria for use in a multicenter phase III vaccine trial. Their report provides the most up-to-date approach to classifying severe malaria on the basis of previously available data.

In this report, we present results of a prospective observational study of clinical and laboratory features among 1,933 children with acute *Plasmodium falciparum* malaria at Mulago Hospital during 2007–2009. We used newer clinical assays, including blood lactate levels, oximetry, and complete blood counts. We present data on the prevalence of major malaria syndromes; the impact of specific syndromes on case-fatality rates; and, for the first time, a cluster analysis of the extent of association between different clinical features present in a large cohort of children with severe malaria. Our findings update the clinical description of severe malaria in children, respond to requests for improved case definitions for severe malaria,[25] and may suggest new research targets and novel treatments for specific sub-groups of patients.

METHODS

Study population. Children 6 months to 12 years of age with either uncomplicated or severe malaria were enrolled in a prospective observational study conducted at the Acute Care Unit of Mulago Hospital in Kampala, Uganda.[26] Mulago Hospital is a 1,500 bed national referral center and teaching hospital of Makerere University College of Health Sciences where a previous study documented a 4.2% case-fatality rate among 23,342 children with malaria.[27] Children were enrolled during October 2007–October 2009. The diagnosis of malaria

*Address correspondence to Walter H. Dzik, Department of Pathology, Blood Transfusion Service, J224, Harvard University, Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114. E-mail: sdzik@partners.org

TABLE 1
World Health Organization features of malaria

Features
Clinical
Impaired consciousness or unrousable coma
Prostration (generalized weakness; unable to walk or sit up without assistance)
Failure to feed
Multiple convulsions (> 2 episodes in 24 hours)
Deep breathing, respiratory distress (acidotic breathing)
Circulatory collapse or shock (systolic blood pressure < 50 mm Hg in children)
Clinical jaundice plus evidence of other vital organ dysfunction
Hemoglobinuria
Abnormal spontaneous breathing
Pulmonary edema (radiologic)
Laboratory findings
Hypoglycemia (blood glucose level < 2.2 mM)
Metabolic acidosis (plasma bicarbonate level < 15 mM)
Severe normocytic anemia (hemoglobin level < 5 g/dL)
Hemoglobinuria
Hyper-parasitemia (> 2% or 100,00 parasites/μL in low-intensity transmission areas or > 5% or 250,00 parasites/μL in areas of high, stable malaria transmission intensity
Hyper-lactatemia (lactate level > 5 mM)
Renal impairment (serum creatinine level > 265 μmol/L)

was suspected on the basis of clinical symptoms and a positive thick blood smear examined by an experienced laboratory technician, and subsequently confirmed by two expert reviewers from a reference parasitology laboratory who examined in a blinded fashion thick and thin blood smears from each person. Uncomplicated malaria was defined as the absence of any impairment of consciousness or hypoxia, with peripheral blood lactate levels < 5 mM and hemoglobin (Hb) levels > 7 g/dL without transfusion. Severe malaria was defined as impaired consciousness, arterial oxygen saturation < 90%, blood lactate levels > 5 mM, or an Hg level < 5 g/dL (or < 6 g/dL if tested after transfusion). Children who did not meet the above criteria for either uncomplicated or severe malaria were not enrolled in the study so that analysis would contrast the spectrum of malaria severity.

All enrolled persons were tested for infection with human immunodeficiency virus (HIV);[26] forty-five were positive and were excluded from the analysis so that the clinical description would represent the effects of malaria alone. For each child, a parent or guardian provided written informed consent for participation in accordance with guidelines of the research ethics committees of the Makerere University College of Health Sciences and the University of Toronto.

Data collection. Two physicians (CM and AD) experienced in malaria care of children enrolled, evaluated, and recorded all information. All available clinical resources were used to assess the presence of coexisting bacterial infection or other medical conditions. Commercially available devices were used to measure complete blood count (ACT*8; Beckman Coulter, Brea, CA), blood lactate (LactatePro LT-1710; Arkray, Kyoto, Japan), oxygen saturation (Nonin, Plymouth, MN), and glucose (Ascensia Contour; Bayer HealthCare LLC, Mishawaka, IN). The presence of Hb S was tested by using a commercial solubility assay (SickleDex; Streck, Omaha, NE). ABO and rhesus blood grouping was determined by using commercial reagents according to manufacturer's directions. Quantitative parasite counts were determined by two independent observers counting the number of parasitized erythrocytes indexed to 200 leukocytes and then corrected for the actual leukocyte count.[26] Structured clinical data for each person were collected in a uniform fashion by using a case report form (CRF) (www.cd36malaria.org). Data from the hard-copy CRF was transferred to a digital CRF (prepared with FileMaker Pro 9.0 version 1; FileMake, Santa Clara, CA) for subsequent analysis. Data accuracy and quality control were performed as reported.[26]

Severe malaria categories. On the basis of their clinical and laboratory results, children with severe malaria were assigned to one or more of the following categories: severe malaria anemia Hb level < 5 g/dL (or < 6 g/dL after transfusion); lactic acidosis: blood lactate level > 5 mM; severe thrombocytopenia: platelet count < 50,000/μL; leukocytosis: total leukocyte count > 10,000 cells/μL; hyper-parasitemia: > 5% of erythrocytes parasitized; hypoxia: peripheral oxygen saturation < 90% while breathing ambient air; and hypoglycemia: blood glucose level < 2.2 mM.

We categorized persons as having CM if they met both of the following two criteria. First, the patient had coma or a Blantyre Coma Scale ≤ 2 provided that the coma was present for > 6 hours and was not attributable to hypoglycemia, meningitis, non-malaria-related pre-existing neurologic abnormalities, or drugs such as anticonvulsants or other agents with sedative/hypnotic effects. Second, the patient met either or both of the following two severity criteria: the patient had > 3 of the following 10 World Health Organization severity criteria: 1) > 2 seizures in 24 hours, RD, jaundice, hemoglobinuria, spontaneous bleeding, hypoglycemia (glucose level < 2.2 mM), LA (lactate level > 5 mM), normocytic severe anemia, hyper-parasitemia >5%, or new acute renal failure; or 2) the patient had a cumulative score of ≥ 3 points on a previously reported scale of neurologic involvement.[26]

Statistical analysis. Continuous data are reported as a median with inter-quartile ranges (IQRs), and were compared by using the Wilcoxon test. Categorical data were compared using the chi-square test. All comparisons were two-tailed and a P value < 0.05 was considered significant. Associations between pairs of categories of severe malaria are presented as odds ratios. Logistic regression was used to determine the odds ratios for the outcome of death using input terms found to have significant association with death in 2 × 2 analysis or known to have a published biologic relationship to adverse outcomes in malaria: presence of CM, hypoxia, severe thrombocytopenia, leukocytosis, LA, hyper-parasitemia, SMA, Hb S, blood group A, age < 1.5 years, and female sex. Of the 855 children with severe malaria, 798 had recorded values for the above 11 input terms and formed the basis for the regression. The enrollment of approximately 1,000 uncomplicated and 1,000 severe malaria patients was designed to detect a difference of ≥ 6% with 80% power between uncomplicated and severe malaria patients for clinical features with a prevalence of 25–50%.

Ethics. The study was approved by the Makerere University School of Medicine Research Ethics Committee, the Toronto Academic Health Science Network Research Ethics Board, and the Uganda National Council for Science and Technology. The study was registered at www.clinicaltrials.gov as NCT00707200.

RESULTS

A total of 2,092 children six months to 12 years of age with either uncomplicated malaria or severe malaria were

enrolled. After study completion, 159 were excluded, leaving 1,933 available for analysis. Reasons for exclusion (specified before the study) were: HIV positivity (n = 45), not infected with *P. falciparum* (n = 35), and not meeting pre-study definitions for uncomplicated or severe disease (n = 79). Illness was attributed exclusively to malaria in nearly all children. For example, among those categorized as having CM (n = 174), one-third (n = 56) had a lumbar puncture performed and none of these children showed evidence of meningitis. Only 38 children received antibiotics for unconfirmed but suspected coexisting bacterial infections. Levels of parasitized erythrocytes were > 2,500/μL in 94% of children[28] and > 5,000/μL in 91%.[24]

All patients were treated by pediatricians expert in malaria care. Intravenous quinine was used in 99% of children with severe malaria. Intravenous hydration, oxygen, and anti-seizure medications were used as needed. Transfusion therapy was readily available. Among 653 patients for whom blood was requested for transfusion, only one failed to receive a transfusion, three received fewer than the prescribed units, and 29 experienced some delay before the start of transfusion because of blood availability.

Clinical and laboratory features of 1,933 children are shown in Table 2. Of these children, 1,078 were classified as having uncomplicated malaria and 855 children were classified as having severe malaria on the basis of enrollment features of neurologic involvement, SMA, LA, or hypoxia. In addition to these enrollment features, children with severe malaria differed from those with uncomplicated malaria for 17 other clinical or laboratory findings. The age distribution of children is shown in Figure 1A. Severe malaria was more common among children < 1.5 years of age.

Clinical and laboratory features among the 855 children with severe malaria are shown in Table 3. The prevalence of findings for each of eight major clinical factors is shown.

Cerebral malaria (n = 174). Hypoglycemia was excluded as a cause of impaired consciousness in nearly all (93%) children categorized as having CM. Patients with CM were distinct from those with SMA; only 36 (4%) of 855 patients had both syndromes. As reported,[23] children with CM were significantly older (median age = 2.5 years, IQR = 1.5–3.9 years) than those without CM (median age = 1.7 years, IQR = 1.0–2.9 years) ($P < 0.0001$) (Figure 1B). Among 855 patients with any form of SM, those with CM had a higher median Hb level (6.9 g/dL, IQR = 5.2–8.2 g/dL versus 4.2 g/dL, IQR = 3.4–4.9 g/dl; $P < 0.0001$) and a lower median platelet count (73,000/μL, IQR = 43,000–129,500/μL versus 110,000/μL, IQR = 66,000–170,000/μL; $P < 0.0001$) than children without CM.

Respiratory distress (n = 518) and lactic acidosis (n = 481). The presence of labored or deep breathing, nasal flaring,

TABLE 2

Clinical and laboratory features among 1,933 children with uncomplicated or severe *Plasmodium falciparum* malaria, Kampala, Uganda*

Characteristic	Uncomplicated malaria, n = 1,078		Severe malaria, n = 855		
	Value	No.	Value	No.	*P*
History and physical examination					
Age, years (range)	2.9 (1.6–5.1)	1,078	1.8 (1.1–3.1)	855	< 0.0001
Body mass index (range)	15.4 (14–17)	797	14.8 (13.6–16.5)	655	< 0.0001
Sex (F:M)	521:557	1,078	402:453	855	0.60
Days ill before hospitalization (range)	3 (2–4)	1,078	3 (3–5)	855	< 0.0001
Temperature, °C (range)	38.2 (37.3–39)	660	37.8 (37.1–38.6)	492	< 0.0001
Patients with palpable spleen	157 (23%)	676	310 (62%)	504	< 0.0001
Patients with respiratory distress	74 (7%)	1,078	518 (61%)	855	< 0.0001
Jaundice	19 (4.1%)	460	88 (26.5%)	332	< 0.0001
Coma	0 (0%)	1,078	200 (23%)	855	NA
Recurrent seizures	0 (0%)	1,078	196 (23%)	855	NA
Blantyre coma score (range)	ND	ND	4 (4–5)	844	NA
Laboratory values upon presentation, median (IQR)					
Hemoglobin (g/dL)	9.3 (8.2–10.4)	1,078	4.5 (3.6–6.3)	855	NA
MCV (fL)	84 (78–89)	1,077	84 (78–90)	855	0.61
Platelet count ($\times 10^9$/L)	136 (81–217)	1,078	103 (60–170)	854	< 0.0001
Leukocyte count ($\times 10^9$/L)	7.8 (5.9–10.3)	1,072	11.1 (7.7–16.7)]	853	< 0.0001
Absolute monocyte count ($\times 10^9$/L)	0.5 (0.3–0.8)	1,065	0.8 (0.5–1.4)	847	< 0.0001
Parasitized erythrocytes/μL ×1,000	83 (29–190)	1,063	91 (22–263)	831	0.13
% erythrocytes parasitized	2.2 (0.8–5.0)	1,062	4.6 (1.2–12.9)	831	< 0.0001
Hemoglobin S (%)	57 (6)	1,045	43 (5)	826	0.89
Glucose (mM)	5 (4.2–6)	65	5 (4.2–6.2)	248	0.65
Lactate (mM)	2.2 (1.6–3.0)	1,052	5.6 (3.1–8.3)	851	NA
Oximetry saturation (%)	99 (97–100)	1,052	97 (94–99)	849	NA
No. patients (%) with specific malaria syndromes					
Cerebral malaria	0 (0)	1,078	174 (20)	855	NA
Lactic acidosis (> 5 mM)	0 (0)	1,052	482 (56)	851	NA
Severe malaria anemia (hemoglobin < 5 g/dL)	0 (0)	1,078	558 (65)	855	NA
Platelets < 50,000/μL	104 (10)	1,078	166 (19)	854	< 0.0001
Leukocytosis (leukocytes > 10,000/μL)	286 (27)	1,072	490 (57)	855	< 0.0001
Hyper-parasitemia (> 5% infected erythrocytes)	264 (25)	1,063	402 (48)	831	< 0.0001
Blood group A or AB	302 (28)	1,078	317 (37)	855	< 0.0001
Hypoxia (SaO_2 < 90%)	0 (0)	1,052	43 (5)	849	NA
Hypoglycemia (< 2.2 mM)	0 (0)	65	22 (8.9)	248	< 0.0001
Death	0 (0)	1,078	48 (4.5)	855	< 0.0001

*Patients were categorized as having uncomplicated or severe malaria on the basis of neurologic findings, hemoglobin levels, blood lactate levels and oxygen saturation (see Methods). NA = not applicable, feature defined enrollment category; ND = not determined; MCV, mean corpuscular volume; IQR, interquartile range; SaO2, arterial oxygen saturation.

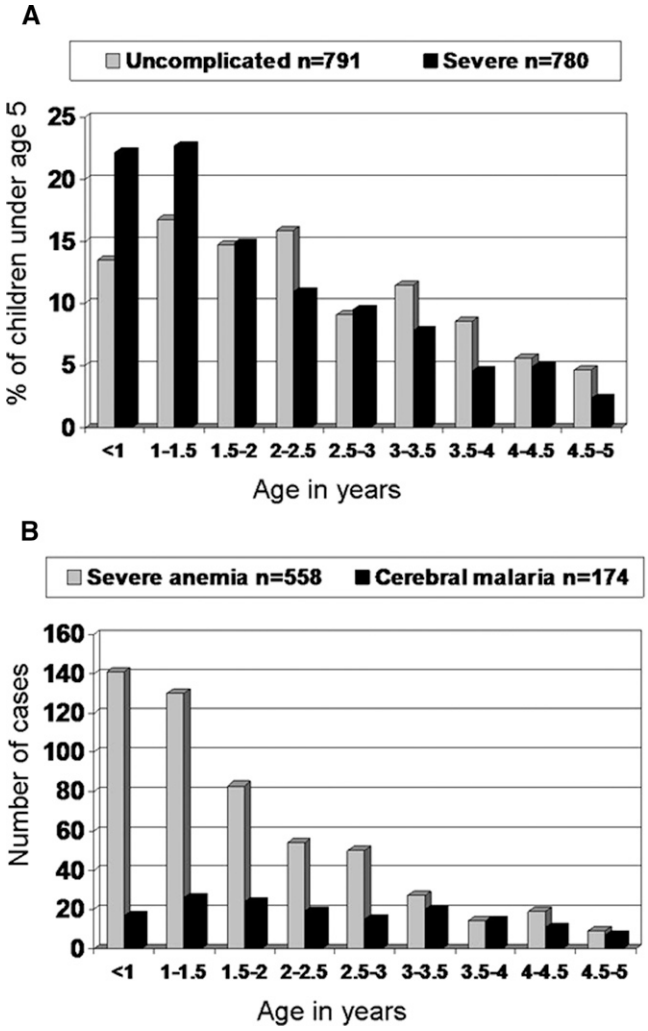

FIGURE 1. Age distribution of children les than five years of age with severe malaria, Kampala, Uganda. **A**, Age distribution among children with uncomplicated versus severe malaria. **B**, Age distribution among children with cerebral malaria or severe anemia.

intercostal or subcostal retractions, or tachypnea (rate > 40 breaths/minute) was directly related to disease severity but was not caused by hypoxia. Respiratory distress was observed in 61% of children with SM, but only 7% of those with uncomplicated malaria (Table 2). Hypoxia, defined as an arterial oxygen saturation < 90%, was observed in only 42 (8%) of 513 patients with RD.

Rather than hypoxia, RD was highly associated with LA (χ^2 = 113, P < 0.0001). Specifically, among 516 children with RD, the median lactate level was 6.85 mM (IQR = 4.5–10.4 mM); 71% had lactate levels > 5 mM and 94% had levels > 2 mM. These results are consistent with those of previous investigators, who suggested that RD represents a respiratory compensation to LA, rather than respiratory drive from hypoxia or lung disease.[8,11] Overall, among 1,903 children tested for blood lactate levels, 481 (25%) had levels > 5 mM. Lactic acidosis was found in 272 (49%) of 556 patients with SMA and in an additional 209 children (70%) with severe malaria without SMA.

Severe malaria anemia (n = 558). Severe malaria anemia was observed in 65% of children with severe malaria. As noted by others,[23] patients with SMA were younger (Figure 1B). Children with SMA had a slightly higher prevalence of splenomegaly (68% versus 51%; P < 0.0001) than those without SMA. Children with SMA also had higher absolute monocyte counts (median = 1,020/μL, IQR = 600–1,580/μL) than those without SMA (median = 547/μL, IQR = 319–886/μL) (P < 0.0001).

Severe thrombocytopenia (n = 166). Thrombocytopenia at admission was a strong indicator of disease severity (median platelet count = 103,000/μL in patients with severe malaria versus 136,000/μL in patients with uncomplicated disease) (P < 0.0001). The proportion of children with a platelet count < 50,000/μL was nearly twice as high (19%) among those with severe syndromes than among those with uncomplicated malaria (10%) (χ^2 = 38, P < 0.0001). The number of patients with CM trebled with platelet counts < 100,000/μL, suggesting that 100,000/μL may be a more informative threshold definition for severe thrombocytopenia in malaria (Figure 2).

Hyper-parasitemia (n = 402). The concentration of parasitized erythrocytes varied widely, and the median concentration was not statistically different between children with uncomplicated disease and those with severe disease. However, as shown in Table 2, the proportion of children with > 5% parasitized erythrocytes was significantly higher among those with severe malaria (48%) than among those with uncomplicated malaria (25%) (P < 0.0001). Nevertheless, the presence of hyper-parasitemia had a positive predictive value of only 60% for severe malaria, and the absence of hyper-parasitemia had a negative predictive value of only 65% for severe malaria. Because the definition of hyper-parasitemia depends on the ratio of infected erythrocytes to total erythrocytes, the presence of anemia increases the likelihood of being classified as hyper-parasitemic for any given absolute concentration of parasitized erythrocytes per microliter of whole blood.

Leukocytosis (n = 490). The median leukocyte count was significantly higher in children with severe malaria (11,100/μL) than in those with uncomplicated malaria (7,800/μL) (P < 0.0001) (Table 2). Consistent with a host inflammatory response to severe disease, a leukocyte count > 10,000/μL was found in 66% of those with SMA, 67% of those with hypoxia, and 77% of those with hypoglycemia (Table 3).

Case-fatality rate. The CFR was significantly different among patients with different severe malaria clinical features (Table 3). The most striking difference was the low CFR (2.8%) among patients with SMA than in those with CM (19%) or severe thrombocytopenia (14.5%). There were 48 deaths attributed to malaria. Major clinical features were found in the following percentages in fatal cases: LA in 79%, CM in 69%, hyper-parasitemia in 62%, severe thrombocytopenia in 50%, SMA in 31%, hypoglycemia in 33%, and hypoxia in 23%.

The relationship between CFR and the number of features present in the same patient are shown in Figure 3. The CFRs progressively increased with an increasing number of the following hallmark features of malaria: CM, LA, SMA, severe thrombocytopenia, and hyper-parasitemia. Logistic regression was used to determine the odds ratios of a fatal outcome according to the following 11 input variables: sex, age < 1.5 years, CM, LA, SMA, severe thrombocytopenia,

TABLE 3

Clinical and laboratory features among 855 children with severe *Plasmodium falciparum* malaria, Kampala, Uganda*

	Cerebral malaria, n = 174		Lactic acidosis (lactate > 5 mM), n = 481		Anemia (hemoglobin < 5 g/dL), n = 558		Thrombocytopenia (< 50,000 platelets/μL), n = 166		Leukocytosis (> 10,000 leukocytes/μL), n = 490		Hyper-parasitemia (> 5% infected erythrocytes), n = 402		Hypoxia (< 90% SaO_2), n = 43		Hypoglycemia (< 2.2 mM), n = 22	
Characteristic	Value	No.	Value	No.	Value	No.	Value	No.	Value	No.	Value	No.	Value	No.	Value	No.
History and physical examination																
Age, years	2.5 (1.5–3.9)	174	1.70 ([1.1–3.0)	481	1.55 (1.0–2.6)	558	2.48 (1.4–4.0)	166	1.48 (1–2.5)	490	1.6 (1.1–2.9)	402	1.92 (1.0–3.1)	43	1.93 (1.3–3.3)	22
BMI	14.7 (13.3–16.7)	122	14.8 (13.7–16.5)	364	14.8 (13.6–16.2)	439	15.0 (13.8–16.5)	112	14.7 (13.5–16.3)	382	14.7 (13.4–16.6)	296	14.4 (13–16)	31	14 (12.7–16)	17
BMI lowest quartile (< 13.6)	39 (32)	122	86 (24)	364	113 (26)	439	25 (22)	112	102 (27)	382	79 (27)	296	9 (29)	31	7 (41)	17
Sex (F:M)	90:84	174	233:248	481	263:295	558	80:86	166	237:253	490	195:207	402	18:25	43	13:9	22
Days ill	3 (3–4)	174	3 (3–5)	481	4 (3–5)	558	3 (3–4)	166	4 (3–5)	490	3 (3–4)	402	4 (3–5)	43	3.5 (3–4)	22
Temperature, °C	37.9 (37.3–38.6)	82	38.0 (37.2–38.7)	301	37.8 (37–38.5)	321	37.9 (37.4–38.4)	102	37.8 (37–38.5)	294	37.9 (37.2–38.7)	245	37.8 (37–38.6)	22	37.8 (37.1–37.9)	11
Palpable spleen (%)	48 (55)	87	187 (60)	311	223 (68)	326	60 (57)	105	205 (68)	300	157 (63)	250	16 (70)	23	7 (64)	11
Respiratory distress (%)	125 (72)	174	367 (76)	481	339 (61)	558	112 (67)	166	342 (70)	490	286 (71)	402	42 (98)	43	21 (95)	22
Jaundice (%)	12 (20)	60	59 (27)	220	61 (28)	213	20 (27)	75	62 (31)	203	41 (24)	170	5 (31)	16	4 (40)	10
Coma (%)	171 (98)	174	103 (22)	470	56 (10)	558	65 (40)	164	98 (20)	487	99 (25)	402	14 (33)	43	17 (77)	22
Seizures (%)	135 (78)	174	113 (24)	470	65 (11.6)	558	55 (33)	164	105 (22)	487	103 (26)	402	15 (35)	43	11 (50)	22
Blantyre coma score	2 (2–2)	174	5 (4–5)	470	5 (5–5)	558	4 (2–5)	164	5 (4–5)	487	5 (4—5)	402	4 (4–5)	43	2 (2–3.75)	22
Patients with CM (%)			79 (16)	481	36 (6.4)	558	57 (34)	166	80 (16)	490	85 (21)	402	10 (23)	43	14 (64)	22
Laboratory values upon presentation																
Lactate (mM)	4.3 (2.7–8.1)	171	8 (6.2–11.1)	481	4.9 (3.0–9.0)	556	7.0 (4.6–10.2)	165	6.05 (3.3–9.9)	488	6.4 (4.1–9.7)	401	9.7 (5.0–12.3)	43	10.0 (6.9–13.4)	22
Patients with lactate > 5 mM (%)	79 (47)	171			272 (49)	556	121 (73)	165	295 (60)	488	267 (67)	401	32 (74)	43	19 (86)	22
Hemoglobin, g/dL	6.9 (5.2–8.2)	174	4.6 (3.4–6.8)	481	3.8 (3.2–4.4)	558	5.9 (4.3–7.7)	166	4.1 (3.3–5)	490	4.6 (3.6–6.5)	402	4.6 (3.4–7.4)	43	4.75 (3.9–7.5)	22
Patients with hemoglobin ≤ 5 g/dL, (%)	36 (21)	174	272 (56)	481			69 (42)	166	370 (76)	490	253 (63)	402	23 (53)	43	12 (55)	22
MCV (fL)	84 (79–89)	174	83.5 (78–90)	481	85.5 (78–92)	558	84.4 (78–89)	166	83 (77–90)	490	84 (78–90)	402	84 (78–89)	43	82 (79–88)	22
Platelet ($\times 10^9$/L)	73 (43–130)	174	90 (49–151)	481	120 (78–179)	558	34 (25–42)	166	118 (73–186)	490	85 (50–129)	402	92 (51–161)	43	90.5 (40–166)	22
Patients with platelet counts < 50,000/μL, (%)	57 (33)	174	121 (25)	481	69 (12)	558			68 (14)	490	100 (25)	402	11 (26)	43	8 (36)	22
Leukocytes ($\times 10^9$/L)	9.4 ([7.1–14.3)	174	11.5 (8.1–18.2)	480	12.6 (8.5–18.8)	556	8.3 (5.4–13.9)	166	15.4 (12.2–21)	490	11.5 (8–17.4)	402	13.6 (9.5–20)	43	16 (10.4–21.2)	22
Patients with leukocyte counts > 10,000/μL, (%)	80 (46)	174	295 (61)	481	370 (66)	558	68 (41)	166			250 (62)	402	29 (67)	43	17 (77)	22
Monocytes ($\times 10^9$/L)	0.6 (0.3–0.9)	174	0.81 (0.5–1.4)	480	1.02 (0.6–1.6)	556	0.46 (0.3–0.8)	166	1.2 (0.8–1.8)	490	0.82 (0.5–1.4)	402	0.74 (0.5–1.3)	43	0.86 (0.7–1.3)	22
Infected erythrocytes/μL ($\times$1,000)	124 (26–387)	171	137 (38–371)	471	68 (15–199)	538	197.5 (46–486)	162	91 (22–274)	480	269 (161–518)	402	137 (22–455)	41	151 (68–508)	21
% Infected erythrocytes	4.6 ([1.1–16.2)	171	7.0 (1.9–16.8)	471	4.2 (0.9–11.9)	538	7.6 (1.9–18.0)	162	5.5 (1.4–15.5)	480	13.6 (8.2–29.8)	402	6.3 (1.1–17.8)	41	7.7 (3.2–22.7)	21
Patients with > 5% infected erythrocytes (%)	85 (50)	171	267 (57)	471	253 (47)	538	100 (62)	162	250 (52)	480			22 (54)	41	14 (64)	21
Hemoglobin S	6 (4)	167	16 (3.4)	465	30 (5.5)	541	5 (3.1)	161	28 (5.9)	474	11 (2.8)	389	3 (7.1)	42	0 (0)	22
Patients with blood type A or AB	71 (41)	174	183 (38)	480	206 (37)	558	59 (35.8)	165	182 (37)	490	146 (36.3)	402	15 (36)	43	8 (36)	22
SaO_2 saturation	96 (94–98)	171	96.5 (94–99)	477	97 (94–99)	555	96 (94–98)	163	97 (94–99)	486	96 (94–99)	398	84 (77–88)	43	95 (91–98)	21
Patients with SaO_2 < 90%, (%)	10 (6)	171	32 (6.7)	476	23 (4.2)	554	11 (6.8)	163	29 (6)	485	22 (5.5)	397			4 (19)	21
Glucose (mM)	5.1 (4.3–6.9)	161	4.9 (3.7–6.1)	124	4.4 (3.6–5.4)	102	5.2 (4.2–6.8)	70	4.6 (3.6–5.4)	123	5.05 (4.1–6.4)	114	3.7 (2.2–7.8)	17	1.35 (0.9–1.9)	22
Patients with glucose < 2.2 mM, (%)	14 (8.7)	161	19 (15)	124	12 (11.8)	102	8 (11.4)	70	17 (13.8)	123	14 (12.3)	114	4 (24)	17		
Deaths (%)	33 (19)	174	37 (7.7)	481	15 (2.7)	558	24 (14.5)	166	33 (6.7)	490	29 (7.2)	402	10 (23.3)	43	10 (45)	22

*Values are medians (interquartile range [IQR]) or no. (%). For example, in the first column, there were 174 children with cerebral malaria. Of these children, body mass index (BMI) values were recorded for 122. The median BMI was 14.7 (IQR = 13.3–16.7) and 39 (32%) of 122 had BMI values < 13.6. SaO2, arterial oxygen saturation; C = cerebral malaria.

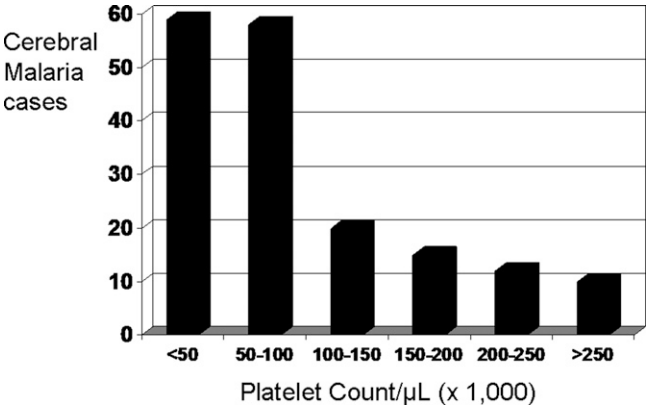

FIGURE 2. Cerebral malaria according to platelet count at presentation, Kampala. Uganda.

leukocytosis, hyper-parasitemia, hypoxia, blood group A, and presence of Hb S. Five factors had significant associations with fatal outcome in the final model: CM, hypoxia, severe thrombocytopenia, leukocytosis, and LA. Test results for interactions among these five factors were found to be not significant. The results are shown in Table 4.

Inter-relationships of malaria syndromes. Inter-relationships between major clinical features of severe malaria are shown in Table 5 and Figure 4. Clinical findings were assembled into clusters on the basis of statistically significant positive odds ratios. Two clusters of associations emerged. In the first cluster, SMA, splenomegaly, and leukocytosis demonstrated mutually significant positive associations of similar magnitude. In the second cluster, seven features demonstrated significant positive inter-relationships. Strong associations centered on the triad of death, severe thrombocytopenia, and LA. Cerebral malaria was associated with death and severe thrombocytopenia; hypoxia and hypoglycemia were associated with death and LA; and hyper-parasitemia was associated with LA and severe thrombocytopenia.

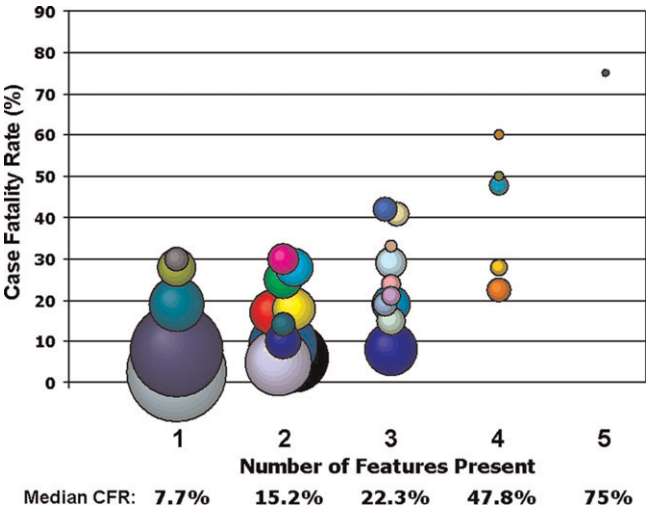

FIGURE 3. Increase in case-fatality rate (CFR) with increasing number of severe malaria features, Kampala, Uganda. CFRs are shown for 855 children with one or more combinations of the following features: cerebral malaria, lactic acidosis, severe anemia, severe thrombocytopenia, or hyper-parasitemia. The x-axis separates children into five groups based on an increasing number of co-existing severe malaria features present in combination. The groups show an increasing median CFR. The size of each bubble indicates the number of persons ranging from n = 558 for the single feature of severe anemia (lower left) to n = 4 for all five features simultaneously present (upper right).

TABLE 4

Logistic regression for fatal outcome based on 798 children with severe malaria, Kampala, Uganda*

Characteristic	Odds ratio	95% Confidence interval	*P*
Cerebral malaria (CM)	10.9	4.8–25.0	< 0.0001
Hypoxia	6.9	2.5–19.1	0.0002
Severe thrombocytopenia	3.8	1.7–8.2	0.0008
Leukocytosis	3.0	1.3–6.9	0.0129
Lactic acidosis (LA)	2.4	1.0–5.5	0.0454
Blood group A	1.8	0.9–3.9	0.1077
Female sex	1.2	0.6–2.5	0.5930
Age < 1.5 years	1.1	0.5–2.5	0.8158
Hemoglobin S	1.0	0.2–6.1	0.9691
Hyper-parasitemia	0.9	0.4–1.8	0.6980
Severe malaria anemia (SMA)	0.7	0.3–1.5	0.3466

Characteristic	Odds ratio	95% Confidence interval	*P*
Cerebral malaria (CM)	13.1	6.2–27.7	< 0.0001
Hypoxia	6.9	2.6–18.8	0.0001
Severe thrombocytopenia	3.6	1.7–7.5	0.0008
Leukocytosis	2.4	1.1–5.3	0.0303
Lactic acidosis (LA)	2.4	1.1–5.4	0.0351

*For the upper panel, Y = 2.4 × CM + 1.9 × Hypoxia + 1.3 × Thrombocytopenia + 1.1 × Leukocytosis + 0.9 × LA – 5.86. For the lower panel, Y = 2.6 × CM + 1.9 × Hypoxia + 1.3 × Thrombocytopenia + 0.9 × Leukocytosis + 0.9 × LA – 5.70. Upper panel shows the odds ratios and 95% confidence intervals for 11 input features of severe malaria. (Model χ^2 = 96.7, degrees of freedom = 11, $P < 0.0001$). Lower panel shows the results for the five statistically significant features. (Model χ^2 = 92.9, degrees of freedom – 5, $P < 0.0001$). CM, hypoxia, severe thrombocytopenia, leukocytosis, LA, hyper-parasitemia, and SMA were entered as dichotomous values as defined in the Methods.

DISCUSSION

Using a standardized assessment, we have analyzed the clinical features at hospitalization of 1,933 children with acute malaria at Mulago Hospital in Kampala, Uganda. We confirmed results of previous reports that SMA affects younger children and CM affects older children with malaria; that LA is found both in association with SMA and independent of SMA; and that RD was unrelated to hypoxia. Our data update existing information on risk factors associated with fatal outcomes in severe malaria.

The three largest recent studies on presenting features in malaria are those of Dzeing-Ella and others,[15] Issifou and others,[16] and Ranque and others,[17] each of which enrolled children more than a decade ago. Our study agrees with the findings of those reports but includes a larger number of children with severe malaria. In addition, we recorded oxygen saturations, measured blood lactate levels for > 10 times as many children, and were able to analyze the independent contributions of thrombocytopenia and leukocytosis to outcomes. Regarding fatal outcomes, we confirm previous findings by many investigators that CM is the principal cause of malaria death; that SMA has a low risk of death if transfusions are available; that CFRs increase in proportion to increasing numbers of co-existing severe malaria features; and that LA and hypoglycemia are associated with fatal outcomes. The CFR for children with CM (19%) was similar to that reported by Marsh and

TABLE 5
Associations between clinical syndromes among children with severe *Plasmodium falciparum* malaria, Kampala, Uganda*

Characteristic	CM	LA	SMA	Severe thrombocytopenia	Leukocytosis	Hyper-parasitemia	Hypoxia	Hypo-glycemia	Splenomegaly
Death	10.39	2.727	0.221	4.684	1.685	1.682	6.156	4.518	0.164
	$P < 0.0001$	$P = 0.003$	$P < 0.0001$	$P < 0.0001$	$P = 0.13$	$P = 0.10$	$P < 0.0001$	$P = 0.002$	$P < 0.0001$
Splenomegaly	0.728	0.846	2.265	0.795	2.034	1.088	1.451	1.578	
	$P = 0.19$	$P = 0.39$	$P < 0.0001$	$P = 0.31$	$P < 0.0001$	$P = 0.71$	$P = 0.51$	$P = 0.54$	
Hypo-glycemia	0.940	7.117	1.8133	1.512	3.849	2.4	3.837		
	$P = 1.0$	$P < 0.001$	$P = 0.25$	$P = 0.46$	$P = 0.007$	$P = 0.07$	$P = 0.045$		
Hypoxia	1.214	2.352	0.596	1.479	1.590	1.263			
	$P = 0.56$	$P = 0.017$	0.1	$P = 0.32$	$P = 0.20$	$P = 0.52$			
Hyper-parasitemia	1.069	2.152	0.858	1.96	1.423				
	$P = 0.73$	$P < 0.0001$	$P = 0.3$	$P < 0.0001$	$P = 0.014$				
Leukocytosis	0.562	1.454	2.903	0.439					
	0.0001	$P < 000.1$	$P < 0.0001$	$P < 0.0001$					
Severe thrombocytopenia	2.556	2.490	0.268						
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$						
SMA	0.081	0.394							
	$P < 0.0001$	$P < 0.0001$							
LA	0.609								
	$P = 0.004$								

* For each combination, odds ratio and *P* value are shown. CM = cerebral malaria; LA = lactic acidosis; SMA = severe malaria anemia.

FIGURE 4. Inter-relationships of clinical and laboratory findings in 855 children with severe malaria, Kampala, Uganda. The odds ratios for association between pairs of clinical and laboratory findings were determined for 855 children with severe malaria. Those features with a statistically significant positive odds ratio of association are shown. The reciprocal of the $\log_e$ of the odds ratio defines the relative distance between spheres, and the number of persons with each feature defines the volume of each sphere. Two clusters of associations were observed. **A**, Cluster centered on severe malaria anemia (SMA). **B**, Cluster of seven features. CM = cerebral malaria; LA = lactic acidosis. Thrombocytopenia = platelet count < 50,000/μL.

others in 1995,[2] suggesting little therapeutic advance for this deadly syndrome. We extend existing reports by identifying with logistic regression five factors associated with fatal outcomes: CM, hypoxia, severe thrombocytopenia, leukocytosis, and LA.

The presence of severe thrombocytopenia was a clinically important finding in our study with prognostic significance. Children with severe malaria had lower median platelet counts than those with uncomplicated malaria (Table 2). In logistic regression analysis, death was 3.6 times more likely in the presence of severe thrombocytopenia. Recent interest has focused on the finding by McMorran and others[29] that growth of *P. falciparum in vitro* was inhibited by co-culture with platelets. However, their non-flow, co-culture system was unable to assess the role of platelets in the cytoadhesion of parasitized erythrocytes to endothelium. Our clinical data support the view that thrombocytopenia is associated with poor outcomes[30] and are consistent with the hypothesis that platelets actively participate in the pathophysiology of cytoadhesion in malaria.[26,31–36]

As shown in Figure 4, we determined inter-relationships among the major clinical features of SM. We observed two clusters of relationships, one cluster in children with SMA, and a second cluster centered on death, severe

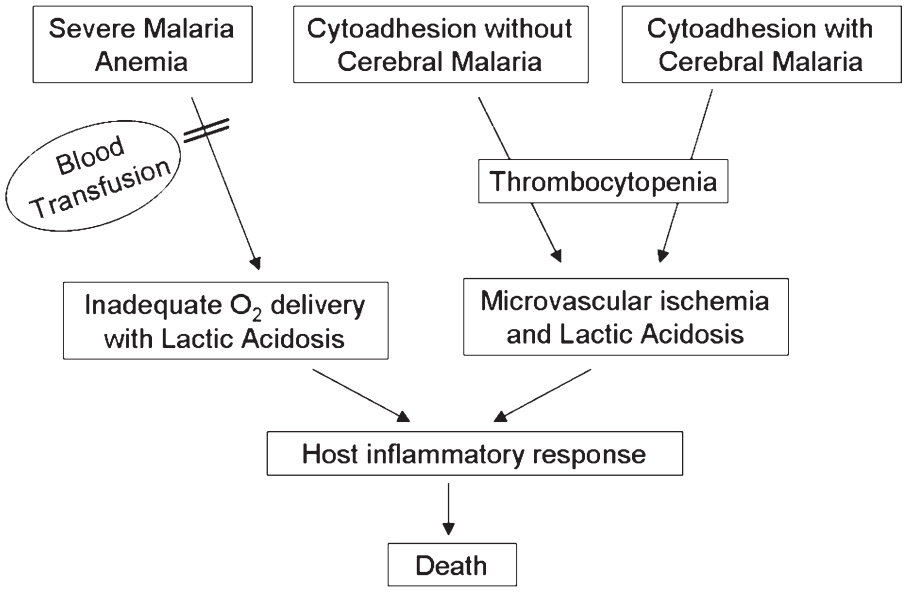

FIGURE 5. Possible pathophysiologic pathways in fatal *Plasmodium falciparum* malaria, Kampala, Uganda. The inter-relationships of clinical features of malaria and the identification of factors with significant odds ratios for fatal outcomes suggest distinct pathophysiologic pathways in children with severe disease.

thrombocytopenia, and LA. These inter-relationships are consistent with the three original major syndromes described by Marsh and others[2] (CM, SMA, and RD) and with three potential pathophysiologic pathways shown in Figure 5. One pathway emphasizes anemia that accompanies some patients with malaria. With blood transfusion, children with SMA can be rescued and fatal outcomes averted.[6] Without transfusion, severe anemia will result in insufficient tissue oxygenation, LA, and RD. A second pathway emphasizes cytoadhesion and microvascular ischemia in the central nervous system resulting in CM. In our dataset, severe thrombocytopenia was strongly associated with CM (Figure 2, Figure 4, and Table 5), suggesting an important role for platelet-mediated cytoadhesion in the cerebral vasculature as suggested by several authors.[31,32,34,37] A third pathway, also directly associated with severe thrombocytopenia, is systemic LA in the absence of CM or SMA (Figure 4 and Table 5). Lactic acidosis with accompanying RD presumably results from microvascular tissue ischemia outside the central nervous system, and in severe cases is associated with hypoglycemia and death. Further research to identify which host or parasite factors favor cytoadhesion in the cerebral vasculature versus the non-cerebral circulation is expected to be of value in guiding new therapies.

Our study had the following limitations. Results are based only on children who were hospitalized. Thus, our data do not reflect general prevalence rates for children at risk for malaria. Serial laboratory data and clinical follow-up data were not collected. We did not collect data for renal function, levels of malaria pigment found in leukocytes, cytokines, retinal examination in all patients with suspected CM or the relative distribution of parasite maturity in peripheral blood. However, none of these features was considered essential in the clinical assessment of malaria by a recent panel of experts.[24]

In summary, we update presenting features of pediatric malaria on the basis of a prospective, uniform, clinical and laboratory assessment of approximately 2,000 children treated at an urban medical center in Uganda. Our data emphasize the clinical distinction between uncomplicated and severe malaria, report the prevalence of cardinal features that characterize syndromes of severe malaria, quantify clustered inter-relationships among malaria syndromes, and identify the major risk factors for fatal outcomes. We hope that these results will not only assist in the care of children with malaria, but may also prove valuable in the planning and assessment of future research.

Received October 30, 2012. Accepted for publication December 21, 2012.

Published online January 28, 2013.

Note: Supplemental video appears at www.ajtmh.org.

Acknowledgments: We thank all pediatric patients and their families who agreed to participate in this study; the staff of the Molecular Biology Laboratory of the University of Makerere University–University of California San Francisco (Dr. Sammuel Nsobya and the parasitology technologists); Dr. Francis Ssali (Joint Center for Clinical Research, Kampala); Dr. Sarah Kiguli-Walube (Department Head of Paediatrics at Makerere University College of Health Sciences); Dr. Robert Opoka (Medical Director of Acute Care Unit, Mulago Hospital); Jolly Rubambarama (Head registered nurse at the Acute Care Unit of Mulago Hospital); the malaria blood film screening staff at the Acute Care Unit (Edson Sabuni, Josephine Birungi, Rehema Namwanje, Timothy Pande, David Balamusani, Stephen Ikodi, Moses Kizito, and Vincent Sekibala); the specimen transport chain management in Kampala (Abdu Mwanje); Dr. Dorothy Kyeyune (Director of the Uganda National Blood Transfusion Service; HIV testing laboratory staff (Dr. Tony Mazzulli and Lilian Law at Mount Sinai Hospital in Toronto) for their contributions to this study; Avogadro, an open source molecular builder for providing a visualization tool, version 1.1.0 (http://avogadro.openmolecules.net/), which was used to prepare the digital model in Figure 4; Eileen Selogie (Enet Answers) (http://www.enetanswers.com/) for developing the animation for public viewing; and Masimo Corporation, Whatman Corporation, Bayer, Ortho Clinical Diagnostics, Nonin Corporation, and Heart to Heart International for providing equipment and supplies.

Disclaimer: Christine M. Cserti-Gazdewich and Walter H. Dzik conceived and designed the study, analyzed data, and prepared the

manuscript. Aggrey Dhabangi, Charles Musoke enrolled patients and collected primary data. Isaac Ssewanyana, Henry Ddungu, Deborah Nakiboneka-Ssenabulya, Nicolette Nabukeera-Barungi, and Arthur Mpimbaza participated in the organization and design of the study, provided oversight, and assisted in data collection. All authors approved the final manuscript.

Financial support: This study was supported by the International Society of Blood Transfusion, the National Blood Foundation, the University of Toronto Dean's Fund, and the Evelyn and Robert Luick Fund.

Authors' addresses: Christine M. Cserti-Gazdewich, Department of Laboratory Hematology, Blood Transfusion Medicine Laboratory, and Department of Medicine and Hematology, University Health Network/Toronto General Hospital, Toronto, Ontario, E-mail: christine.cserti@uhn.ca. Aggrey Dhabangi, Child Health and Development Centre, Kampala, Uganda, E-mail: adhabangi@gmail.com. Charles Musoke and Nicolette Nabukeera-Barungi, Department of Paediatrics and Child Health, Makerere University College of Health Sciences/Mulago Hospital, Kampala, Uganda, E-mails: jxyug@yahoo.com and nicbarungi@yahoo.com. Isaac Ssewanyana, Central Public Health Laboratories, Kampala, Uganda, E-mail: sewyisaac@yahoo.co.uk. Henry Ddungu, Uganda Cancer Institute, Mulago Hill, Kampala, Uganda, and the African Palliative Care Association, Makindye Hill, Kampala, Uganda, E-mail: hddungu@gmail.com. Deborah Nakiboneka-Ssenabulya, London School of Hygiene and Tropical Medicine, London, UK, E-mail: debsenabulya@yahoo.com. Arthur Mpimbaza, Child Health and Development Centre, Makerere University College of Health Sciences/Mulago Hospital, Kampala, Uganda, E-mail: arthurwakg@yahoo.com. Walter H. Dzik, Department of Pathology, Blood Transfusion Service, J224, Harvard University, Massachusetts General Hospital, Boston, MA, E-mail: sdzik@partners.org.

REFERENCES

1. World Health Organization, 2010. *Guidelines for the Treatment of Malaria.* Geneva: World Health Organization.
2. Marsh K, Forster D, Waruiru C, Mwangi I, Winstanley M, Marsh V, Newton C, Winstanley P, Warn P, Peshu N, 1995. Indicators of life-threatening malaria in African children. *N Engl J Med 332:* 1399–1404.
3. Marsh K, Snow RW, 1997. Host-parasite interaction and morbidity in malaria endemic areas. *Philos Trans R Soc Lond B Biol Sci 352:* 1385–1394.
4. Waller D, Krishna S, Crawley J, Miller K, Nosten F, Chapman D, ter Kuile FO, Craddock C, Berry C, Holloway PA, 1995. Clinical features and outcome of severe malaria in Gambian children. *Clin Infect Dis 21:* 577–587.
5. White NJ, Miller KD, Marsh K, Berry CD, Turner RC, Williamson DH, Brown J, 1987. Hypoglycaemia in African children with severe malaria. *Lancet 1:* 708–711.
6. Lackritz EM, Campbell CC, Ruebush TK II, Hightower AW, Wakube W, Steketee RW, Were JB, 1992. Effect of blood transfusion on survival among children in a Kenyan hospital. *Lancet 340:* 524–528.
7. Slutsker L, Taylor TE, Wirima JJ, Steketee RW, 1994. In-hospital morbidity and mortality due to malaria-associated severe anaemia in two areas of Malawi with different patterns of malaria infection. *Trans R Soc Trop Med Hyg 88:* 548–551.
8. Krishna S, Waller DW, ter Kuile F, Kwiatkowski D, Crawley J, Craddock CF, Nosten F, Chapman D, Brewster D, Holloway PA, 1994. Lactic acidosis and hypoglycaemia in children with severe malaria: pathophysiological and prognostic significance. *Trans R Soc Trop Med Hyg 88:* 67–73.
9. Taylor TE, Borgstein A, Molyneux ME, 1993. Acid-base status in paediatric *Plasmodium falciparum* malaria. *Q J Med 86:* 99–109.
10. Molyneux ME, Taylor TE, Wirima JJ, Borgstein A, 1989. Clinical features and prognostic indicators in paediatric cerebral malaria: a study of 131 comatose Malawian children. *Q J Med 71:* 441–459.
11. English M, Waruiru C, Amukoye E, Murphy S, Crawley J, Mwangi I, Peshu N, Marsh K, 1996. Deep breathing in children with severe malaria: indicator of metabolic acidosis and poor outcome. *Am J Trop Med Hyg 55:* 521–524.
12. English M, Waruiru C, Marsh K, 1996. Transfusion for respiratory distress in life-threatening childhood malaria. *Am J Trop Med Hyg 55:* 525–530.
13. English M, Sauerwein R, Waruiru C, Mosobo M, Obiero J, Lowe B, Marsh K, 1997. Acidosis in severe childhood malaria. *QJM 90:* 263–270.
14. Schellenberg D, Menendez C, Kahigwa E, Font F, Galindo C, Acosta C, Schellenberg JA, Aponte JJ, Kimario J, Urassa H, Mshinda H, Tanner M, Alonso P, 1999. African children with malaria in an area of intense *Plasmodium falciparum* transmission: features on admission to the hospital and risk factors for death. *Am J Trop Med Hyg 61:* 431–438.
15. 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, 2005. Severe falciparum malaria in Gabonese children: clinical and laboratory features. *Malar J 4:* 1.
16. Issifou S, Kendjo E, Missinou MA, Matsiegui PB, Dzeing-Ella A, Dissanami FA, Kombila M, Krishna S, Kremsner PG, 2007. Differences in presentation of severe malaria in urban and rural Gabon. *Am J Trop Med Hyg 77:* 1015–1019.
17. Ranque S, Poudiougou B, Traore A, Keita M, Oumar AA, Safeukui I, Marquet S, Cabantous S, Diakite M, Mintha D, Cisse MB, Keita MM, Dessein AJ, Doumbo OK, 2008. Life-threatening malaria in African children: a prospective study in a meso-endemic urban setting. *Pediatr Infect Dis J 27:* 130–135.
18. Idro R, Karamagi C, Tumwine J, 2004. Immediate outcome and prognostic factors for cerebral malaria among children admitted to Mulago Hospital, Uganda. *Ann Trop Paediatr 24:* 17–24.
19. Idro R, Ndiritu M, Ogutu B, Mithwani S, Maitland K, Berkley J, Crawley J, Fegan G, Bauni E, Peshu N, Marsh K, Neville B, Newton C, 2007. Burden, features, and outcome of neurological involvement in acute falciparum malaria in Kenyan children. *JAMA 297:* 2232–2240.
20. Mockenhaupt FP, Ehrhardt S, Burkhardt J, Bosomtwe SY, Laryea S, Anemana SD, Otchwemah RN, Cramer JP, Dietz E, Gellert S, Bienzle U, 2004. Manifestation and outcome of severe malaria in children in northern Ghana. *Am J Trop Med Hyg 71:* 167–172.
21. Jarvis JN, Planche T, Bicanic T, Dzeing-Ella A, Kombila M, Issifou S, Borrmann S, Kremsner PG, Krishna S, 2006. Lactic acidosis in Gabonese children with severe malaria is unrelated to dehydration. *Clin Infect Dis 42:* 1719–1725.
22. Newton CR, Valim C, Krishna S, Wypij D, Olola C, Agbenyega T, Taylor TE, 2005. The prognostic value of measures of acid/base balance in pediatric falciparum malaria, compared with other clinical and laboratory parameters. *Clin Infect Dis 41:* 948–957.
23. Roca-Feltrer A, Carneiro I, Smith L, Schellenberg JR, Greenwood B, Schellenberg D, 2010. The age patterns of severe malaria syndromes in sub-Saharan Africa across a range of transmission intensities and seasonality settings. *Malar J 9:* 282.
24. Vekemans J, Marsh K, Greenwood B, Leach A, Kabore W, Soulanoudjingar S, Asante KP, Ansong D, Evans J, Sacarlal J, Bejon P, Kamthunzi P, Salim N, Njuguna P, Hamel MJ, Otieno W, Gesase S, Schellenberg D, 2011. Assessment of severe malaria in a multicenter, phase III, RTS, S/AS01 malaria candidate vaccine trial: case definition, standardization of data collection and patient care. *Malar J 10:* 221.
25. Anstey NM, Price RN, 2007. Improving case definitions for severe malaria. *PLoS Med 4:* e267.
26. Cserti-Gazdewich CM, Dhabangi A, Musoke C, Ssewanyana I, Ddungu H, Nakiboneka-Ssenabulya D, Nabukeera-Barungi N, Mpimbaza A, Dzik WH, 2012. Cytoadherence in paediatric malaria: ABO blood group, CD36, and ICAM1 expression and severe *Plasmodium falciparum* infection. *Br J Haematol 159:* 223–236.
27. Opoka RO, Xia Z, Bangirana P, John CC, 2008. Inpatient mortality in children with clinically diagnosed malaria as compared with microscopically confirmed malaria. *Pediatr Infect Dis J 27:* 319–324.
28. Bejon P, Berkley JA, Mwangi T, Ogada E, Mwangi I, Maitland K, Williams T, Scott JA, English M, Lowe BS, Peshu N, Newton

CR, Marsh K, 2007. Defining childhood severe falciparum malaria for intervention studies. *PLoS Med 4:* e251.
29. McMorran BJ, Marshall VM, de Graaf C, Drysdale KE, Shabbar M, Smyth GK, Corbin JE, Alexander WS, Foote SJ, 2009. Platelets kill intraerythrocytic malarial parasites and mediate survival to infection. *Science 323:* 797–800.
30. Gerardin P, Rogier C, Ka AS, Jouvencel P, Brousse V, Imbert P, 2002. Prognostic value of thrombocytopenia in African children with falciparum malaria. *Am J Trop Med Hyg 66:* 686–691.
31. Pain A, Ferguson DJ, Kai O, Urban BC, Lowe B, Marsh K, Roberts DJ, 2001. Platelet-mediated clumping of *Plasmodium falciparum*-infected erythrocytes is a common adhesive phenotype and is associated with severe malaria. *Proc Natl Acad Sci USA 98:* 1805–1810.
32. Wassmer SC, Lepolard C, Traore B, Pouvelle B, Gysin J, Grau GE, 2004. Platelets reorient *Plasmodium falciparum*-infected erythrocyte cytoadhesion to activated endothelial cells. *J Infect Dis 189:* 180–189.
33. Wassmer SC, Taylor T, Maclennan CA, Kanjala M, Mukaka M, Molyneux ME, Grau GE, 2008. Platelet-induced clumping of *Plasmodium falciparum*-infected erythrocytes from Malawian patients with cerebral malaria-possible modulation *in vivo* by thrombocytopenia. *J Infect Dis 197:* 72–78.
34. Bridges DJ, Bunn J, van Mourik JA, Grau G, Preston RJ, Molyneux M, Combes V, O'Donnell JS, de Laat B, Craig A, 2010. Rapid activation of endothelial cells enables *Plasmodium falciparum* adhesion to platelet-decorated von Willebrand factor strings. *Blood 115:* 1472–1474.
35. Mayor A, Hafiz A, Bassat Q, Rovira-Vallbona E, Sanz S, Machevo S, Aguilar R, Cistero P, Sigauque B, Menendez C, Alonso PL, Chitnis CE, 2011. Association of severe malaria outcomes with platelet-mediated clumping and adhesion to a novel host receptor. *PLoS ONE 6:* e19422.
36. Phiri HT, Bridges DJ, Glover SJ, van Mourik JA, de Laat B, M'Baya B, Taylor TE, Seydel KB, Molyneux ME, Faragher EB, Craig AG, Bunn JE, 2011. Elevated plasma von Willebrand factor and propeptide levels in Malawian children with malaria. *PLoS ONE 6:* e25626.
37. Cox D, McConkey S, 2010. The role of platelets in the pathogenesis of cerebral malaria. *Cell Mol Life Sci 67:* 557–568.