Am. J. Trop. Med. Hyg., 96(5), 2017, pp. 1176–1184
doi:10.4269/ajtmh.16-0315

Clinical Comparison of Retinopathy-Positive and Retinopathy-Negative Cerebral Malaria

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Abstract. Cerebral malaria (CM) is a severe and often lethal complication of falciparum malaria. A classic malaria retinopathy is seen in some (retinopathy-positive [RP]) children but not others (retinopathy-negative [RN]), and is associated with increased parasite sequestration. It is unclear whether RN CM is a severe nonmalarial illness with incidental parasitemia or a less severe form of the same malarial illness as RP CM. Understanding the clinical differences between RP and RN CM may help shed light on the pathophysiology of malarial retinopathy. We compared clinical history, physical examination, laboratory findings, and outcomes of RP (N = 167) and RN (N = 87) children admitted to Mulago Hospital, Kampala, Uganda. Compared with RN children, RP children presented with a longer history of illness, as well as physical examination and laboratory findings indicative of more severe disease and organ damage. The hospital course of RP children was complicated by longer coma duration and a greater transfusion burden than RN children. Mortality did not differ significantly between RP and RN children (14.4% versus 8.0%, P = 0.14). Further, severity of retinal hemorrhage correlated with the majority of variables that differed between RP and RN children. The data suggest that RP and RN CM may reflect the spectrum of illness in CM, and that RN CM could be an earlier, less severe form of disease.

INTRODUCTION

Malaria remains a leading global health concern, with an estimated 214 million cases of malaria worldwide in 2015 and an estimated 438,000 deaths, mostly in sub-Saharan Africa (90%) and in children under 5 years of age (70%).[1] Cerebral malaria (CM) is the most severe neurological complication of falciparum malaria, and is associated with a case fatality rate (CFR) of up to 15%, whereas 24% of survivors develop neurocognitive sequelae such as attention and memory deficits.[2,3]

The pathogenesis of coma and mechanisms that lead to death in CM remain unknown. One important pathophysiologic feature of CM is the sequestration of mature parasitized red blood cells (pRBCs) in the microvasculature of the brain.[4] Although the brain is not accessible during examination, the retina is a potential proxy given the many similarities in the vessel network and tissue embryology.[5] Examination of the ocular fundus in children with CM has revealed a characteristic retinopathy that has both diagnostic and prognostic significance.[6,7] This unique retinopathy is characterized by retinal hemorrhages, vessel changes, and retinal whitening. Retinal hemorrhages have been shown to correlate with brain hemorrhages on autopsy.[8] Vessel changes and discoloration have been associated with sequestration of pRBCs on histopathological examination, and retinal whitening has been shown to be caused by local tissue hypoxia.[9,10] Additionally, the presence and severity of malarial retinopathy (MR) is associated with parasite sequestration in the brain, prolonged coma, and death.[7,11,12]

The inability to exclude other causes of coma in a parasitemic child may lead to overdiagnosis of CM in malaria-endemic regions. In one prospective autopsy study in Malawi, 23% of children with clinically diagnosed CM were found to have died of other causes, and MR was the only clinical sign to distinguish malarial from nonmalarial cause of death.[6] Previous studies in Malawi have revealed that retinopathy-positive (RP) children have higher rates of respiratory distress and lower hematocrits compared with retinopathy-negative (RN) children and that RN CM children have a shorter febrile prodrome and lower mortality,[12,13] but it remains unclear whether RN CM is a severe nonmalarial illness with incidental parasitemia or a less severe form of the same malarial illness as RP CM. Additionally, MR is found in other types of severe malaria. In a recent study conducted in Ghana, MR was seen in 73% of children with CM and 54% of children with severe malarial anemia.[14] Although prior studies have assessed the differences between specific clinical and laboratory factors such as hematocrit and presence of respiratory distress in children with CM with and without MR, no study to date has conducted a comprehensive comparison of the full range of symptoms, physical findings, laboratory findings, and outcomes in children with CM who do or do not have MR. Such an evaluation would provide a better assessment of the extent to which pathophysiology differs in the two groups, and provide guidance on whether the children with MR CM appear to have a similar but less severe clinical syndrome, or a different clinical syndrome entirely.

For this reason, in the present study, we conducted a comprehensive analysis of potentially relevant clinical symptoms, signs, laboratory values, and outcomes in children with CM who did and did not have MR.

METHODS

Study population. The study was conducted at Mulago Hospital, Kampala, Uganda, from 2008 to 2013. Children with CM were enrolled if they were between 18 months and 12 years of age. CM was defined as coma (Blantyre coma scale score $\leq$ 2), *Plasmodium falciparum* on blood smear, and no other known cause of coma (e.g., meningitis, a prolonged postictal state, or hypoglycemia). Exclusion criteria included cerebrospinal fluid (CSF) white blood cells (WBC) > 5 cells/μL, positive CSF culture, known chronic

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illness requiring medical care, known developmental delay, or history of coma, head trauma, hospitalization for malnutrition, or cerebral palsy.

Clinical assessment. During their initial presentation, all children underwent a standardized medical history and physical examination. On enrollment, patients received an initial blood draw including a blood culture, and a lumbar puncture was performed if parents or guardian consented and child was clinically stable enough to perform the lumbar puncture. Children with CM were managed per the Ugandan Ministry of Health treatment guidelines current at the time of the study. These included intravenous quinine treatment followed by oral quinine for severe malaria during hospital admission and artemisinin-based combination therapy for outpatient follow-up therapy. Glucose, blood transfusions, antibiotics, and anticonvulsants were administered if clinically indicated.

Children were assessed for MR by means of indirect ophthalmoscopy. An ophthalmologist experienced in the evaluation of MR trained the two senior study investigators (Chandy C. John and Robert O. Opoka), a study ophthalmologist, as well as study medical officers in identifying and recording findings of MR. The study investigators and ophthalmologist then continued training and assessing the study medical officers for accuracy in this assessment and recording of ophthalmoscopic findings, and interim retraining and assessment was done by Simon Harding, a second expert in malaria retinopathy. Ophthalmology evaluation was done by medical officers in all CM patients on admission, and repeated every 24 hours while they remained comatose. Before each examination, pupils were dilated with sequential instillation of cyclopentolate 1% and tropicamide 1%. Using a binocular indirect ophthalmoscope, an eye examination was performed 30–60 minutes later. The findings were noted on case report forms with record made of the presence and severity of retinal hemorrhages, vessel changes, and retinal whitening. Children were considered to have MR if at least one of these findings was present.

Laboratory testing. Peripheral blood smears were assessed for *Plasmodium* species by microscopy with Giemsa staining according to standard protocols. Two independent readings were conducted and if not consistent, were resolved by a third reading. For stool examination, stool was collected from the patients, and 10 samples from each specimen were emulsified with saline and examined for evidence of intestinal parasites by trained laboratory technologists. Plasma bilirubin, glucose, haptoglobin, lactate dehydrogenase (LDH), blood urea nitrogen (BUN), and creatinine levels were measured by the Advanced Research and Diagnostic Laboratory at the University of Minnesota. Plasma *P. falciparum* histidine-rich protein-2 (*Pf*HRP-2) levels were quantified using the commercially available Malaria Ag CELISA kit (Cellabs, Brookvale, Australia), as described previously.[15]

Statistical analysis. Data was analyzed using Stata/SE 13.1 for Windows (StataCorp, College Station, TX). A Pearson's χ^2 test was used to test for associations between categorical variables and retinopathy, unless expected cell frequencies were below 5, when Fisher's exact test was used. The Student's *t* test was used to compare means of normally distributed data. For nonnormally distributed continuous variables, the Kruskal–Wallis test was used. Spearman bivariate analysis was used to correlate the severity of retinal hemorrhage with continuous variables that had statistically significant differences between RP and RN children. For significantly different categorical variables, odds ratios and the *P* value for trend across groups were calculated with logistic regression. $P < 0.05$ was regarded as significant.

No adjustment was made for multiple comparisons; however, for *P* values > 0.001, exact numbers are reported so that Bonferroni–Dunn corrections can be calculated (i.e., $\alpha = 0.05/k$, where k = number of tests).

Ethics statement. Ethical approval was granted by the institutional review boards for human studies at Makerere University School of Medicine and the University of Minnesota. Written consent was obtained from parents or guardians of study participants.

RESULTS

Retinopathy findings. During the period of study, a total of 267 children with CM were enrolled. Of the 267 children with CM, 13 did not have funduscopic examination, yielding a total of 254 children with CM included in this analysis. Children were classified into two groups based on funduscopic findings: RP ($N = 167$) and RN ($N = 87$). Over the course of hospitalization, 26 patients (15.6%) had at least one discordant examination. Six patients (3.6%) had positive examinations that became negative, 14 patients (8.4%) had negative examinations that became positive, and six patients (3.6%) had examinations with multiple different positive or negative findings. Of the 167 children with retinopathy, 139 had retinal hemorrhages, 60 had vessel changes, 86 had macular whitening, and 60 had peripheral whitening. In the same group of children, 71 had one feature of retinopathy, 41 had two features, 25 had three features, and 30 had all four features of retinopathy.

A total of 54 children had repeat examinations by an ophthalmologist within 24 hours of the initial examination, and 18.5% examinations were discordant between the two readings.

Demographic characteristics. The age range was from 1.5 to 11.7 years, with a mean age (standard deviation) of 3.91 (1.94) years. Of the 254 CM patients who had funduscopic examination, 149 (59%) were males. Age, sex, and time to first ophthalmologic assessment were not significantly different in CM children with and without retinopathy ($P > 0.2$ for all, Table 1).

History. In the history of their present illness, RP CM children had longer mean coma duration, longer mean duration of breathing difficulty, and longer mean time since last meal than RN CM children ($P < 0.035$ for all, Table 2), suggesting a longer history of illness in RP children. A higher percentage of RP CM children had difficulty breathing and had used antimalarials prior to presenting to the hospital as compared with RN CM children ($P < 0.05$ for both, Table 2). There were no significant differences in the past medical history questions listed in Table 2 ($P > 0.05$ for all).

Examination findings. In the initial medical examination, RP CM children had significantly higher mean heart rate, higher mean respiratory rate, and higher rates of hypoxia

TABLE 1
Comparison of demographic characteristics and time to first ophthalmologic assessment between groups categorized by eye findings

	RP (N = 167)	RN (N = 87)	P value*
Mean age (years) (SD)	3.80 (1.97)	4.12 (1.88)	0.217
Male (%) (95% CI)	57.5 (49.6–65.1)	60.9 (49.9–71.2)	0.598
Mean weight-for-age z-score (SD)	–1.29 (1.23)	–1.13 (1.53)	0.352
Mean height-for-age z-score (SD)	–0.54 (1.41)	–0.38 (1.42)	0.413
	N = 152	N = 83	
Median time to first ophthalmologic assessment (hours) (IQR)	3.0 (2.0–5.5)	2.5 (1.5–4.3)	0.208

CI = confidence interval; IQR = interquartile range; RN = retinopathy negative; RP = retinopathy positive; SD = standard deviation. For tests that did not include all participants, the n is listed below the result.
*For categorical data, the χ^2 test was used to compare the two groups. For data represented with mean and SD, the t test was used. For data represented with median and IQR, the Kruskal–Wallis test was used.

than children with RN CM ($P < 0.05$ for all, Table 3). RP children were also more likely to have nasal flaring, retractions, chest indrawing, pallor, jaundice, and a palpable liver than RN CM children ($P < 0.05$ for all, Table 3), indicating more severe disease at presentation in RP CM children.

Laboratory findings. The RP CM children had significantly lower hemoglobin and haptoglobin levels, and higher LDH levels as compared with RN children at enrollment ($P < 0.02$, for all, Table 4), suggesting a more severe anemia and hemolysis in children with RP CM. Acute kidney injury (AKI) is a common complication of severe malaria, and markers of AKI have been shown to have prognostic significance.[16,17] RP children had significantly higher BUN and creatinine than RN children, suggesting that children with RP CM have more AKI ($P < 0.04$ for both, Table 4). RP children also have significantly higher BUN/creatinine ratios, suggestive of greater hypovolemia and a prerenal state ($P = 0.006$, Table 4).

Both RP and RN CM children had similarly high peripheral parasite density at enrollment and 24 hours ($P > 0.46$ for both, Table 4), suggesting that the peripheral parasite burden and clearance in the first 24 hours is similar in these children. However, *Pf*HRP-2 levels were significantly higher in children with RP than RN CM ($P = 0.006$, Table 4), as was previously reported in a cohort from Malawi.[18]

We did not find evidence of more coinfections in CM RN children. Both RP and RN CM children had low, but similar prevalence of hookworm infection, presence of parasites in the stool, human immunodeficiency virus positivity, and positive blood cultures (all $P > 0.34$, Table 4), suggesting

TABLE 2
Comparison of initial and past medical history in cerebral malaria children with and without retinopathy

	RP (N = 167)	RN (N = 87)	P value†
Mean duration of coma before presentation (hours) (SD)	17.9 (14.3)	13.8 (11.3)	0.023*
Mean duration of fever before presentation (days) (SD)	3.33 (1.46)	3.10 (1.45)	0.242
Cough (%) (95% CI)	38.9 (31.5–46.8)	40.2 (29.9–51.3)	0.840
Mean duration of cough before presentation (days) (SD)	1.78 (2.95)	2.31 (4.51)	0.260
Difficulty breathing (%) (95% CI)	31.7 (24.8–39.4)	14.9 (8.2–24.2)	0.004*
Mean duration of difficulty breathing before presentation (days) (SD)	0.42 (0.76)	0.22 (0.64)	0.035*
Diarrhea (%) (95% CI)	4.2 (1.7–8.4)	8.0 (3.3–15.9)	0.201
Vomit (%) (95% CI)	35.9 (28.7–43.7)	37.9 (27.7–49.0)	0.753
Tea-colored urine (%) (95% CI)	18.0 (12.5–24.6)	14.9 (8.2–24.2)	0.542
Mean duration of tea-colored urine before presentation (days) (SD)	0.24 (0.60)	0.23 (0.62)	0.876
Convulsions (%) (95% CI)	93.4 (88.5–96.7)	94.3 (87.1–98.1)	0.794
Mean no. of convulsions before presentation (SD)	4.87 (3.94)	4.06 (4.14)	0.119
Mean time since first convulsion (hours) (SD)	18.1 (16.9)	14.4 (12.3)	0.075
Mean time since most recent convulsion (hours) (SD)	5.41 (9.0)	5.78 (8.7)	0.749
Mean time since last ate (hours) (SD)	36.5 (20.0)	27.6 (15.0)	< 0.001*
Mean time since last drank (hours) (SD)	17.1 (14.7)	14.2 (11.1)	0.102
Antimalarials taken prior to presentation (%) (95% CI)	83.2 (76.7–88.7)	72.4 (61.8–81.5)	0.043*
Antibiotics taken prior to presentation (%) (95% CI)	22.9 (16.7–30.0)	27.6 (18.6–38.2)	0.410
Herbal medicine prior to presentation (%) (95% CI)	38.9 (31.5–46.8)	44.8 (34.1–55.9)	0.364
Past medical history			
Prior history of convulsions (%) (95% CI)	21.0 (15.1–27.9)	26.4 (17.6–37.0)	0.324
Family history of convulsions (%) (95% CI)	3.5 (1.3–7.7)	4.6 (1.3–11.4)	0.739
Prior admission to health unit (%) (95% CI)	30.5 (23.7–38.1)	31.0 (21.5–41.9)	0.935
Mean no. of prior admissions (SD)	0.46 (1.1)	0.38 (0.7)	0.532
Treated for being yellow at birth (%) (95% CI)	0.0 (0.0–2.2)	1.1 (0.0–6.2)	0.343
Hospitalized at birth (%) (95% CI)	1.8 (0.4–5.2)	1.1 (0.0–6.2)	1.000
Premature delivery (%) (95% CI)	0.6 (0.0–3.3)	1.1 (0.0–6.2)	1.000
Other problems at birth (%) (95% CI)	1.2 (0.1–4.3)	0.0 (0.0–4.2)	0.548
Delayed in sitting, standing, or walking (%) (95% CI)	2.4 (0.7–6.0)	1.1 (0.0–6.2)	0.663
Difficulty hearing (%) (95% CI)	0.0 (0.0–2.2)	1.1 (0.0–6.2)	0.343
Problems speaking clearly (%) (95% CI)	0.0 (0.0–2.2)	2.3 (0.3–8.1)	0.116
Problems learning how to do things (%) (95% CI)	1.2 (0.1–4.3)	0.0 (0.0–4.2)	0.548

CI = confidence interval; RN = retinopathy negative; RP = retinopathy positive; SD = standard deviation.
*$P < 0.05$.
†For categorical data, the χ^2 test was used to compare the two groups. For data represented with mean and SD, the t test was used.

TABLE 3
Comparison of initial examination in cerebral malaria children with and without retinopathy

	RP (N = 167)	RN (N = 87)	P value†
Mean temperature (°C) (SD)	37.80 (1.07)	37.68 (1.25)	0.437
Mean heart rate (beats per minute) (SD)	151 (27)	144 (28)	0.040*
Mean systolic blood pressure (mm Hg) (SD)	95.4 (12.0)	95.4 (12.9)	0.987
Mean diastolic blood pressure (mm Hg) (SD)	62.3 (11.7)	62.2 (12.4)	0.927
Mean respiratory rate (breaths per minute) (SD)	46.9 (14.2)	42.7 (14.2)	0.024*
Mean O_2 saturation (%) (SD)	95.2 (5.4)	96.1 (5.4)	0.202
O_2 saturation < 95% (%) (95% CI)	31.1 (24.2–38.8)	19.5 (11.8–29.4)	0.049*
Mean Blantyre coma scale (SD)	1.67 (0.50)	1.60 (0.58)	0.357
	N = 129	N = 65	
Blantyre coma scale ≤ 1 (%) (95% CI)	31.0 (23.2–39.7)	35.4 (23.9–48.2)	0.539
	N = 129	N = 65	
Respiratory			
Grunting (%) (95% CI)	11.4 (7.0–17.2)	10.3 (4.8–18.7)	0.803
Nasal flaring (%) (95% CI)	22.2 (16.1–29.2)	11.5 (5.7–20.1)	0.038*
Retractions (%) (95% CI)	18.6 (13.0–25.3)	5.7 (1.9–12.9)	0.004*
Chest indrawing (%) (95% CI)	17.4 (11.9–24.0)	3.4 (0.7–9.7)	0.001*
Deep breathing (%) (95% CI)	9.6 (5.6–15.1)	6.9 (2.6–14.4)	0.470
Irregular breathing (%) (95% CI)	3.0 (1.0–6.8)	1.1 (0.0–6.2)	0.667
Rales (%) (95% CI)	6.6 (3.3–11.5)	6.9 (2.6–14.4)	0.925
Cardiovascular			
Capillary refill > 2 seconds (%) (95% CI)	14.4 (9.4–20.6)	14.9 (8.2–24.2)	0.903
Temperature gradient of legs (%) (95% CI)	44.3 (36.6–52.2)	40.2 (29.9–51.3)	0.533
Dry mucous membranes (%) (95% CI)	19.2 (13.5–26.0)	10.3 (4.8–18.7)	0.070
Pallor (%) (95% CI)	47.3 (39.5–55.2)	21.8 (13.7–32.0)	< 0.001*
Jaundice (%) (95% CI)	58.1 (50.2–65.7)	39.1 (28.8–50.1)	0.004*
Abnormal heart sounds (%) (95% CI)	3.0 (1.0–6.8)	0.0 (0.0–4.2)	0.169
Abdominal			
Liver palpable (%) (95% CI)	68.9 (61.2–75.8)	47.1 (36.3–58.1)	0.001*
Spleen palpable (%) (95% CI)	43.7 (36.1–51.6)	32.2 (22.5–43.1)	0.075

CI = confidence interval; RN = retinopathy negative; RP = retinopathy positive; SD = standard deviation. For tests that did not include all participants, the n is listed below the result.
*$P < 0.05$.
†For categorical data, the χ^2 test was used to compare the two groups. For data represented with mean and SD, the t test was used.

that the RN presentation in this cohort is probably not influenced by or due to another bacterial or parasitic coinfection with incidental parasitemia.

In the present study, a cohort of healthy children from the same extended household or neighborhood as the children with CM had a prevalence of asymptomatic parasitemia of 14.7%, so the likelihood of a child in coma from these areas incidentally having parasitemia should be no greater than this, and under this hypothesis, this percentage of children might have RN CM. Yet, the actual percentage of RN CM was 34.3% of all children with CM.

Electroencephalogram findings. Electroencephalograms (EEGs) were obtained in 166 children with CM, 121 with RP CM and 45 with RN CM. Seven of the 121 children with RP CM had seizure activity on EEG, of whom three had status epilepticus. None of the seven had a clinical seizure at the time of EEG, so 7/121 (5.8%) and 3/121 children with RP CM (2.5%) had subclinical seizure activity or status epilepticus, respectively. Four of 45 children with RN CM had seizure activity on EEG, of whom three had status epilepticus. One of the four children had clinical seizure activity, so subclinical seizures or status epilepticus occurred in 3/45 (6.7%) and 2/45 (4.4%) of children with RN CM, respectively. The proportion of children with subclinical seizure activity or status epilepticus did not differ significantly between children with RP and RN CM (P values all > 0.2).

Outcomes. In our study, the overall mortality rate in children with CM was 12.2%. The CFR was higher in RP than RN children, but this difference was not statistically significant (14.4% versus 8.0%, P = 0.14; Table 5). RP CM children also had longer admission and coma duration after admission than RN CM ($P < 0.001$ for both, Table 5). RP CM children were also more likely to have repeated convulsions, to need blood transfusion, and to receive anticonvulsants as compared with RN children ($P < 0.02$ for all, Table 5).

Retinal hemorrhage severity. The majority of variables with statistically significant differences between RP and RN children had statistically significant correlations with the severity of retinal hemorrhage, with worse disease seen with increasing number of hemorrhages (Table 6, Table 7). These included markers of illness duration, respiratory distress, anemia, parasite biomass, end organ damage, and clinical outcomes.

DISCUSSION

The present study suggests that RP and RN CM represent a spectrum of clinical disease, in which clinical signs and symptoms are similar in RP and RN CM, but more severe in RP CM. Evidence to support this conclusion includes the data that RP children presented with a longer history of illness and had a higher likelihood of prior antimalarial treatment consistent with a longer time of illness that could lead to more severe illness, that they presented with physical examination and laboratory findings indicative of more severe disease, and their hospital course was complicated by a longer coma duration and higher frequency of blood transfusion. Children with RP and RN CM did not have increased CSF WBC that would support a diagnosis of viral or bacterial meningitis or encephalitis, had negative

TABLE 4
Comparison of laboratory findings in cerebral malaria children with and without retinopathy

	RP (*N* = 167)	RN (*N* = 87)	*P* value†
Mean hemoglobin (g/dL) (SD)	6.51 (2.23)	7.75 (2.23)	< 0.001*
Median WBC count (×10^3/μL) (IQR)	9.8 (7.4–14.2) *N* = 163	8.5 (6.3–13.7)	0.133
Median platelet count (×10^3/μL) (IQR)	61 (37–108) *N* = 163	57 (30–111)	0.896
Median LDH (U/L) (IQR)	856 (680–1,146)	712.5 (492–994)	0.003*
Median haptoglobin (mg/dL) (IQR)	0 (0–0)	0 (0–1)	0.019*
Median total bilirubin (mg/dL) (IQR)	1.5 (0.9–2.6)	1.4 (0.7–2.2)	0.214
Median glucose (mmol/L) (IQR)	6.4 (4.9–8.8)	6.4 (4.8–9.3)	0.965
Median lactate (mmol/L) (IQR)	3.7 (2.3–7.1) *n* = 163	3.7 (2.0–6.1) *N* = 76	0.258
Median BUN (mg/dL) (IQR)	18.0 (13.0–26.0) *N* = 165	14.0 (10.0–21.0) *N* = 86	0.002*
Median creatinine (mg/dL) (IQR)	0.43 (0.32–0.55) *N* = 165	0.36 (0.29–0.52) *N* = 86	0.040*
Median BUN/creatinine ratio (IQR)	44.4 (32.3–52) *N* = 159	36.1 (29.3–47.6) *N* = 83	0.006*
Hemoglobin present on urine dipstick (%) (95% CI)	20.9 (14.4–28.6) *N* = 139	16.7 (8.9–27.3) *N* = 72	0.465
Positive blood culture (%) (95% CI)	10.8 (6.4–16.7) *N* = 157	11.0 (5.1–19.8) *N* = 82	0.972
Positive HIV screen (%) (95% CI)	1.3 (0.2–4.6) *N* = 154	3.7 (0.8–10.3) *N* = 82	0.345
Stool positive for parasites (%) (95% CI)	6.3 (2.8–12.0) *N* = 127	4.9 (1.0–13.7) *N* = 61	0.706
Hookworm infection (%) (95% CI)	0.8 (0.0–4.3) *N* = 127	0.0 (0.0–5.9) *N* = 61	1.000
Median CSF WBC count (cells/μL) (IQR)	0 (0–0) *N* = 122	0 (0–0) *N* = 59	0.919
Positive CSF culture (%) (95% CI)	0 (0–0) *N* = 122	0 (0–0) *N* = 61	1.000
Median parasite density at 0 hours (/μL) (IQR)	47,880 (9,920–295,500) *N* = 161	49,765 (15,480–273,100) *N* = 80	0.457
Median parasite density at 24 hours (/μL) (IQR)	2,080 (0–64,180) *N* = 143	1,620 (32–13,990) *N* = 83	0.551
Median *Plasmodium falciparum* HRP-2 (ng/mL)	3,163 (1,510–5,555)	2,308 (470–5,176)	0.006*

BUN = blood urea nitrogen; CSF = cerebrospinal fluid; HIV = human immunodeficiency virus; HRP-2 = histidine-rich protein-2; IQR = interquartile range; LDH = lactate dehydrogenase; RN = retinopathy negative; RP = retinopathy positive; SD = standard deviation; WBC = white blood cell. For tests that did not include all participants, the *n* is listed below the result.
*$P < 0.05$.
†For categorical data, the χ^2 test or Fisher's exact test was used to compare the two groups. For data represented with mean and SD, the *t* test was used. For data represented with median and IQR, the Kruskal–Wallis test was used.

TABLE 5
Comparison of clinical outcomes in cerebral malaria children with and without retinopathy

	RP (*N* = 167)	RN (*N* = 87)	*P* value†
Case fatality rate (%) (95% CI)	14.4 (9.4–20.6)	8.0 (3.3–15.9)	0.144
Median length of admission [if survived] (days) (IQR)	7 (6–8) *N* = 143	6 (5–7) *N* = 80	< 0.001*
Median coma duration (hours) (IQR)	58.0 (32.0–88.0)	38.8 (20.0–56.3)	< 0.001*
Seizure during admission (%) (95% CI)	57.5 (49.6–65.1)	48.3 (37.4–59.2)	0.162
Median no. of seizures during admission (IQR)	1 (0–2)	0 (0–1)	0.073
Repeated convulsions > 2 in 24 hours (%) (95% CI)	68.3 (60.6–75.2)	52.9 (41.9–63.7)	0.016*
Focal seizures during admission (%) (95% CI)	24.6 (18.2–31.8)	14.9 (8.2–24.2)	0.076
Generalized seizures during admission (%) (95% CI)	35.9 (28.7–43.7)	35.6 (25.6–46.6)	0.963
Received transfusion (%) (95% CI)	68.3 (60.6–75.2)	36.8 (26.7–47.8)	< 0.001*
Median no. of transfusions (IQR)	1 (0–1)	0 (0–1)	< 0.001*
Received anticonvulsants (%) (95% CI)	86.2 (80.1–91.1)	69.0 (58.1–78.5)	0.001*
Received IV fluids (%) (95% CI)	32.9 (25.9–40.6)	23.0 (14.6–33.2)	0.099
Abnormal neurological examination on discharge (%) (95% CI)	38.3 (30.2–46.9) *N* = 141	30.0 (20.3–41.3) *N* = 80	0.215
Abnormal neurological examination at 6-month follow-up (%) (95% CI)	7.2 (3.5–12.8) *N* = 139	2.7 (0.3–9.4) *N* = 74	0.224

CI = confidence interval; IV = intravenous; RN = retinopathy negative; RP = retinopathy positive; IQR = interquartile range. For results that did not include all participants, the *n* is listed below the individual result.
*$P < 0.05$.
†For categorical data, the χ^2 test was used to compare the two groups. For data represented with median and IQR, the Kruskal–Wallis test was used.

TABLE 6

Values for clinical variables that differed significantly in retinopathy positive and retinopathy negative children with cerebral malaria, according severity of retinal hemorrhage

No. of retinal hemorrhages	0 (*N* = 115)	1–5 (*N* = 74)	6–20 (*N* = 39)	21–50 (*N* = 16)	> 50 (*N* = 10)	r_s, Spearman *P* value
History						
Mean duration of coma before presentation (hours) (SD)	14.2 (11.5)	18.4 (14.9)	19.4 (16.3)	13.3 (7.9)	22.8 (15.0)	0.125, 0.046*
Mean duration of difficulty breathing before presentation (days) (SD)	0.22 (0.60)	0.45 (0.71)	0.38 (0.67)	0.38 (0.62)	1.00 (1.63)	0.191, 0.002*
Mean time since last ate (hours) (SD)	28.6 (15.9)	37.4 (22.0)	36.1 (17.4)	39.5 (19.9)	40.9 (19.0)	0.232, < 0.001*
Examination						
Mean heart rate (beats per minute) (SD)	144 (28)	155 (28)	150 (23)	153 (29)	143 (25)	0.118, 0.061
Mean respiratory rate (breaths per minute) (SD)	43.1 (14.9)	46.5 (14.3)	46.9 (13.4)	53.1 (12.7)	47.3 (8.6)	0.199, 0.002*
Mean O_2 saturation (%) (SD)	96.2 (4.9)	95.3 (5.4)	95.0 (5.0)	93.6 (7.0)	92.9 (7.6)	–0.147, 0.019*
Laboratory findings						
Mean hemoglobin (g/dL) (SD)	7.68 (2.27)	6.56 (2.14)	6.29 (2.40)	5.86 (1.81)	5.4 (1.55)	–0.323, < 0.001*
Median LDH (U/L) (IQR)	704 (498–967)	914 (705–1,150)	846 (616–1,111)	849 (753–1,172)	1,306 (1,131–1,593)	0.274, < 0.001*
Median haptoglobin (mg/dL) (IQR)	0 (0–2)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	–0.202, 0.001*
Median BUN (mg/dL) (IQR)	14 (10–21) *N* = 114	19 (13–29) *N* = 72	19 (13–31)	17 (15–25)	20 (17–28)	0.238, < 0.001*
Median creatinine (mg/dL) (IQR)	0.37 (0.29–0.52) *N* = 114	0.45 (0.32–0.59) *N* = 72	0.45 (0.33–0.55)	0.36 (0.31–0.47)	0.47 (0.35–0.71)	0.102, 0.106
Median BUN/Cr ratio (IQR)	36.4 (29.1–48.0) *N* = 110	44.7 (30.2–53.6) *N* = 69	45.5 (35.7–52.0) *N* = 38	45.9 (38.8–58.6) *N* = 15	40.2 (32.8–56.0)	0.210, 0.001*
Median *Plasmodium falciparum* HRP-2 (ng/mL) (IQR)	2,308 (516–5,148)	3,347 (1,536–5,376)	2,917 (1,538–5,659)	2,409 (1,727–5,252)	6,263 (3,350–15,314)	0.202, 0.001*
Clinical outcomes						
Median length of admission [if survived] (days) (IQR)	6 (5–7) *N* = 106	7 (5–8) *N* = 67	7 (6–8) *N* = 31	7 (6–9) *N* = 11	7 (6–9) *N* = 8	0.199, 0.003*
Median coma duration (hours) (IQR)	40 (21–64)	52 (32–82)	62 (37–90)	62 (0–90)	90 (46–129)	0.204, 0.001*
Median no. of transfusions (IQR)	0 (0–1)	1 (0–1)	1 (0–1)	1 (1–2)	1 (1–2)	0.324, < 0.001*

BUN = blood urea nitrogen; CI = confidence interval; HRP-2 = histidine-rich protein-2; IQR = interquartile range; LDH = lactate dehydrogenase; SD = standard deviation. For tests that did not include all participants, the *n* is listed below the result.

*$P < 0.05$.

CSF bacterial cultures, and had a similar low frequency of subclinical seizure activity and status epilepticus on EEG, suggesting that bacterial meningitis, viral encephalitis, or subclinical status epilepticus were not major contributors to coma in either group. There was a trend toward higher mortality in the RP group (14.4% versus 8.0%, $P = 0.14$), which may have reached statistical significance with a larger sample size. Finally, many of these variables correlated with the severity of retinal hemorrhage, suggesting a spectrum of disease within RP CM itself. The study findings regarding longer history of illness, greater kidney injury, and the correlation of clinical findings associated with RP CM with severity of retinopathy have not been reported in prior studies. Together with study findings that corroborate those of previous studies showing that many factors are similar in RP and RN CM, differing only in degree, these data provide the best evidence to date that RN CM may be a clinically less severe variant of CM, rather than a different disease (or diseases) altogether.

Previous studies have demonstrated that children with RP CM present with more severe illness, including a study in Malawi in which children with RP CM had a significantly lower hematocrit, longer coma duration, increased prevalence of deep breathing, and a higher CFR than children with RN CM, and another study of Malawian children with RN CM which found that RN children had a shorter febrile prodrome, fewer abnormalities on physical examination, a quicker recovery, and a lower CFR than those reported for RP CM children.[12,13] The present study adds to these studies with new findings providing additional evidence that the hematopoietic, respiratory, neurologic, and renal systems are involved in both forms of CM, but with more severe involvement in RP CM.

Malarial retinopathy correlates with pathologic findings of cerebral sequestration, and the absence of sequestration in children diagnosed with RN CM has led to uncertainty in the etiology of coma and death in these children.[9] Existing hypotheses for the pathophysiologic process of

TABLE 7

Odds ratios of categorical variables that differed significantly in retinopathy-positive and retinopathy-negative children with cerebral malaria, according severity of retinal hemorrhage

No. of retinal hemorrhages	0 (*N* = 115)	1–5 (*N* = 74)	6–20 (*N* = 39)	21–50 (*N* = 16)	> 50 (*N* = 10)	Trend *P* value
History						
Difficulty breathing (OR) (95% CI)	1.00	3.10 (1.52–6.32)	2.40 (1.01–5.66)	2.45 (0.75–8.02)	3.59 (0.90–14.39)	0.011*
Focal seizures before presentation (OR) (95% CI)	1.00	2.20 (0.73–6.69)	4.10 (1.25–13.49)	2.60 (0.47–14.34)	2.02 (0.22–18.88)	0.067
Generalized seizures before presentation (OR) (95% CI)	1.00	0.40 (0.18–0.88)	0.41 (0.16–1.07)	0.55 (0.14–2.22)	1.15 (0.13–9.89)	0.252
Antimalarials taken prior to presentation (OR) (95% CI)	1.00	1.66 (0.79–3.45)	2.40 (0.85–6.79)	2.47 (0.52–11.68)	1.41 (0.28–7.08)	0.087
Examination						
O_2 saturation < 95% (OR) (95% CI)	1.00	1.69 (0.86–3.35)	2.00 (0.88–4.53)	2.40 (0.78–7.40)	4.00 (1.03–15.47)	0.008*
Nasal flaring (OR) (95% CI)	1.00	2.30 (1.07–4.95)	1.46 (0.54–3.91)	3.03 (0.90–10.16)	0.74 (0.09–6.33)	0.266
Retractions (OR) (95% CI)	1.00	2.01 (0.84–4.82)	2.07 (0.73–5.83)	1.35 (0.27–6.78)	4.05 (0.89–18.49)	0.073
Chest indrawing (OR) (95% CI)	1.00	3.87 (1.37–10.96)	4.69 (1.46–15.05)	4.19 (0.91–19.36)	4.54 (0.76–27.07)	0.007*
Pallor (OR) (95% CI)	1.00	3.60 (1.85–7.01)	3.42 (1.54–7.60)	7.92 (2.32–26.98)	5.4 (1.35–21.61)	< 0.001*
Jaundice (OR) (95% CI)	1.00	1.73 (0.96–3.15)	2.23 (1.05–4.76)	6.05 (1.55–23.58)	1.40 (0.38–5.12)	0.006*
Liver palpable (OR) (95% CI)	1.00	1.63 (0.89–3.00)	3.43 (1.41–8.33)	1.95 (0.63–6.02)	0.89 (0.24–3.24)	0.068
Clinical Outcomes						
Case fatality rate (OR) (95% CI)	1.00	1.23 (0.44–3.47)	3.04 (1.06–8.71)	5.35 (1.45–19.70)	2.94 (0.53–16.29)	0.004*
Repeated convulsions > 2 in 24 hours (OR) (95% CI)	1.00	1.88 (1.01–3.53)	1.42 (0.67–3.03)	1.75 (0.58–5.42)	3.19 (0.63–16.00)	0.062
Received transfusion (OR) (95% CI)	1.00	4.23 (2.16–8.27)	4.26 (1.85–9.82)	11.72 (2.30–59.61)	6.70 (1.29–34.79)	< 0.001*
Received anticonvulsants (OR) (95% CI)	1.00	1.38 (0.67–2.85)	2.82 (0.90–8.76)	0.97 (0.29–3.25)	undefined	0.058

CI = confidence interval; OR = odds ratio.
*$P < 0.05$.

RN CM include 1) symptomatic coinfection without cerebral sequestration, 2) severe nonmalarial illness with incidental parasitemia, and 3) a less severe form of the same malarial illness. In our study, there was no significant difference in the prevalence of positive blood cultures or stool microscopy for common parasites—two proposed sources of coinfection with immunomodulating properties. Although we did not do CSF polymerase chain reaction testing for viral infections, the lack of WBC or RBC in the CSF would be atypical in a viral encephalitis (though it can occur), and the lack of a different EEG pattern in children with RN than RP CM also suggests that viral encephalitis is not the major cause of RN CM. Incidental parasitemia also does not fully explain our study findings, as in the present study, prevalence of asymptomatic parasitemia in healthy children from the same households was 14.7%, whereas the percentage of RN CM was 34.3% of all children with CM, more than double the expected "incidental" rate of 14.7%.

The study data most strongly supports the hypothesis that RN and RP CM are at different points in the spectrum of CM, but both caused by *P. falciparum*. The more severe hemolysis seen in RP CM could lead to increased severity of CM. Increased release of cell-free hemoglobin has been shown to lead to impaired nitric oxide availability, which in turn leads to subsequent vasospasm and impaired microvascular function, important pathophysiologic mechanisms in CM.[19,20] Increased work of breathing in RP CM children could also be directly related to increased hemolytic anemia and subsequent tissue hypoxia. Additionally, the increase in focal seizures may be an indicator of a greater burden of focal vascular insults to the brain, including both vasospasm and cerebral sequestration. In a postmortem study of brain tissue in Vietnamese adults who died of CM, cerebral sequestration was associated with deeper levels of coma and shorter time to death.[4] In our study, longer coma duration could be attributed to the increased amount of sequestration seen in children with RP CM as compared with RN CM.[7] The higher levels of *Pf*HRP-2 similarly suggest a higher degree of parasite biomass and potentially sequestration in children with RP as compared with RN CM, and is similar to the findings of an earlier Malawian study.[18] The correlation with severity of retinal hemorrhage further supports the hypothesis that RP and RN CM are on a spectrum of illness, with RN CM likely representing earlier disease without detectable evidence of sequestration by indirect ophthalmoscopy.

Children with RN CM have less evidence of cerebral sequestration,[6] which may suggest an alternate etiology, but blood vessel dysfunction may also be important to the pathophysiology of CM. Spastic constriction of cerebral arterioles has been identified in CM patients.[21] It is possible that children with RN CM have not yet developed cerebral sequestration, but they do experience vasospasm, which results in their symptoms of CM. It is possible, and consistent with our findings of shorter symptom duration in RN CM, that children in the RN CM group presented earlier in the illness, allowing them to receive treatment before developing retinopathy and sequestration. Taken together, these findings support the idea that RN and RP CM share the same disease process.

A limitation of our study was the lack of repeat examinations on every child—resource limits and the stressful nature of repeated in-depth examinations precluded our doing this in all children. In the subgroup of children who were examined by two examiners, there was discordance of 15.6% on examinations, showing that there may have been misclassification of retinopathy findings in a small number of children. Although a study with stronger concordance of findings would have provided even more rigorous evaluation of retinopathy, the training and retraining in our study (from experts from the Malawi center), the ability of our site to do the fundoscopy examinations, and the ability to have cross-checking on a subset of children by an ophthalmologist are all conditions not easily available even at other African research sites. Thus, we believe that our

findings provide a good evaluation of how evaluation of retinopathy might occur in a best-case real-world situation, and thus are of greatest use in considering whether retinopathy as assessed by real-world study clinicians (the ones who would do the examinations if retinopathy were a required criterion for CM) truly documents different syndromes or more likely reflects the spectrum of CM. Additionally, the percentage of RN children in our cohort was similar to that in studies in Malawi that pioneered this evaluation; our group was trained by experts from the Malawi group, and we found a significant difference in *Pf*HRP-2 levels between RP and RN children, as did the Malawi group, all of which would suggest that the eye examinations done in this study were as accurate as can be obtained in this population. However, consistent with the findings of Seydel and others, our findings suggest that *Pf*HRP-2 levels may be a simpler and more accurate way to distinguish true CM from coma due to other causes, if an accurate, reliable, and easy-to-use point-of-care *Pf*HRP-2 measurement device can be developed.

We conclude that RP and RN CM children appear to be on a spectrum of illness rather than etiologically disparate. In our study, RP CM children presented later in their illness, had greater parasite biomass, and had more severe hemolytic anemia, which could lead to more vasospasm. Together, these factors could explain the increased work of breathing and presence of focal seizures in children with RP CM. Based on these findings, we hypothesize that *P. falciparum* is responsible for illness in RN CM, and that the symptoms may begin to appear before cerebral sequestration is present, possibly due to cerebral arteriolar vasospasm or to inflammation. Malaria retinopathy remains a valuable finding with diagnostic and prognostic value, but further research should be done to identify the parasite and host response factors that lead to RP versus RN CM.

Received April 21, 2016. Accepted for publication November 9, 2016.

Published online January 30, 2017.

Acknowledgments: We thank the children and families who participated in the study, as well as the clinical staff at Mulago Hospital. We also thank Susan Lewallen and Simon Harding for training the clinical staff, and John Ssenkusu for assistance with the statistical analysis.

Financial support: This work was supported by the U.S. National Institutes of Health National Institute of Neurological Disorders and Stroke and the Fogarty International Center (R01 NS055349 and D43 NS078280) and the Doris Duke Charitable Foundation through a grant supporting the Doris Duke International Clinical Research Fellows Program at the University of Minnesota. Chandler Villaverde is a Doris Duke International Clinical Research Fellow.

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