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Spleen volume and clinical disease manifestations of severe *Plasmodium falciparum* malaria in African children

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Abstract

Background—*Plasmodium falciparum* malaria is common in African children. Severe disease manifestations include severe malarial anemia (SMA) and cerebral malaria (CM). In vitro studies suggest that splenic sequestration is associated with SMA and protective against CM. We sought to characterize the relationship between ultrasonographically derived spleen volume (SV), clinical manifestations and outcome.

Methods—We conducted a prospective observational study of severe malaria and SV in children aged 3 months to 12 years in Eastern Uganda. An SV normogram was generated from 186 healthy controls and adjusted for total body surface area (TBSA). Children with severe *P. falciparum* malaria were classified according to disease phenotype, and SV z-scores were compared for cases and controls to assess the degree of spleen enlargement.

Results—One hundred and four children with severe malaria, median age 19.2 months, were enrolled; 54 were classified as having SMA and 15 with CM. Mortality was 27% in the CM group vs 1.9% in the SMA group. TBSA-adjusted SV z-scores were lower in children with CM compared to SMA (1.98 [95% CI 1.38–2.57] vs 2.73 [95% CI 2.41–3.04]; $p=0.028$). Mean SV z-scores were lower in children who died (1.20 [95% CI 0.14–2.25]) compared to survivors (2.58 [95% CI 2.35–2.81]); $p=0.004$.

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Authors' contributions: SK and KM conceived the study; SK, JN, POO and SOA designed the study protocol; SK and JN carried out the clinical assessment; SK and CLM carried out the ultrasonographic analysis; SK and KM carried out the data analysis and interpretation; SK, POO and KM drafted the manuscript. All authors read and approved the final manuscript. SK is the guarantor of the paper.

Competing interests: None declared

Ethical approval: The study was approved by the Mbale Regional Hospital Institutional Review Committee (MRHIRC) and the London School of Hygiene & Tropical Medicine, Ethics Committee.

Conclusions—SV is lower in CM compared to SMA. Severe malaria with no increase in SV z-score may be associated with mortality.

Keywords

Africa; Anemia; Falciparum; Malaria; Spleen; Ultrasonography

Introduction

An estimated 3.3 billion people live in areas endemic for *Plasmodium falciparum* malaria. Of the 780 000 deaths that result from malaria infection, 85% occur in children under 5 years, mostly in Sub-Saharan Africa.¹ The burden and clinical spectrum of severe malaria depends on the background level of acquired immunity, which is dependent on the pattern and intensity of malaria transmission.^{2–4} Severe malaria in children is a complex multi-system disorder⁵ however three major manifestations are widely recognized: cerebral malaria (CM), severe malarial anemia (SMA) and respiratory distress.⁶ Where malaria transmission intensity is high (entomological inoculation rate [EIR] >100 per year),⁷ severe disease and secondary immunity are acquired in early childhood.⁸ While incident rates of clinical malaria in hyper-endemic regions are increased, relative malarial morbidity declines rapidly with age at high levels of transmission.⁷ As such, the greatest disease burden is borne by children under 5 years with a shift towards a predominance of the SMA-phenotype in the younger age groups and a lower incidence of CM as the predominant disease phenotype.^{3,4} In areas with low transmission intensity (EIR<10),⁷ severe malaria affects a wider age group and morbidity data demonstrate increased rates of CM under low transmission pressures.^{2–4,9,10}

In areas of stable malaria transmission, childhood spleen rates have been used as an epidemiologic marker of population immunity, exposure and transmission intensity.^{11–15} Clinical immunity develops after repeated exposures and is thought to modulate parasite density and limit severe disease.¹⁶ Studies characterizing the deleterious effects of splenectomy in endemic areas support a significant role of the spleen in malarial immunity.^{17–19} While current knowledge regarding the exact role of the spleen in malarial immunity is limited, it is thought that both phagocytic and cellular immune functions are involved.²⁰ In areas with high transmission intensity, and high rates of splenomegaly, modulation of parasite density results in a semi-immune state. Enhanced parasite clearance in semi-immunes, however, may come at a price. Since splenic clearance of both infected and uninfected erythrocytes is more pronounced in the setting of splenomegaly, this ultimately leads to a reduction in mean red cell mass as measured clinically by hemoglobin.²¹

Whilst characterized in epidemiological surveys, few studies have characterized acute changes in spleen volume (SV) during a new episode of malaria, particularly life threatening disease. The purpose of this study was to describe the association between severe malarial disease phenotype, specifically the manifestation of SMA and CM, and the degree of spleen enlargement measured using ultrasonographically derived SV.

Materials and Methods

During the months of July–September 2010, we conducted a prospective observational study of children presenting with severe malaria to Mbale Regional Referral Hospital (MRRH), Eastern Uganda: an area of high malaria transmission. EIR for the area is not known, although data for nearby regions at similar latitude and elevation report an annual cumulative EIR in the range of 397–1586 infected bites per person per year.²² Basic laboratory services are available and regional blood transfusion services provide whole blood for pediatric transfusion in accordance with national guidelines.

Population

Children aged 3 months to 12 years presenting with a febrile illness (presence or history of fever) with signs or symptoms consistent with severe malaria, as defined by modified WHO clinical or laboratory criteria,²³ and a positive rapid diagnostic test (RDT) for *P. falciparum* malaria (OpitiMAL, Diamed, Switzerland) were eligible for the study. Informed consent was obtained at enrolment from a parent or guardian. The study was approved by the MRRH Institutional Review Committee and the London School of Hygiene & Tropical Medicine Ethics Committee.

Data collection and study protocol

Demographic and clinical data were collected in consecutive cases by trained clinicians using standardized forms at admission. Nutritional status was assessed by height, weight, mid-upper arm circumference (MUAC), and visual assessment of oedema and/or visible severe wasting. Consciousness was assessed using the Blantyre coma scale (BCS) for children 0–4 years and the Glasgow Coma Score (GCS) was used in children aged 5–12 years. Haemoglobin (g/dl) was determined by HemoCue (Angelholm, Sweden) at admission (0 hours), 8 hours and 24 hours. Blood glucose was determined by a handheld glucometer (Acon Labs, San Diego CA, USA) at admission and every 8 hours until the child was conscious and able to take and retain oral feeds. Lactate was determined at admission and 8 hour observation (Lactate Pro, Arkray Labs, Amsterdam, The Netherlands). Treatment of malaria was conducted according to the Uganda national guidelines.²⁴ Children with severe anaemia were transfused with whole blood at a dose of 20 ml/Kg body weight given over 3–4 hours. Fluid administration, including boluses, were given at the discretion of the treating physician. Case definition for severe malaria was defined according to WHO guidelines as malaria positive (slide or RDT) plus one or more of: impaired consciousness, hemodynamic shock, respiratory distress, or severe anaemia. Cerebral malaria was defined as a child with *P. falciparum* positive RDT and unrousable coma (GCS <8, BCS <3) lasting more than 30 min after a seizure and absence of other causes of coma. Severe malarial anemia was defined as a child with *P. falciparum* positive RDT with signs of severe malaria and Hb <5 g/dl. The definition for shock was that which was used in the FEAST trial.²⁵

Splenic biometry

A portable Phillips CX50 Ultrasound System (Phillips Healthcare, Andover, MA, USA) equipped with an S5-1 phased array probe (1–5 MHz) and a C5-1 curvilinear probe (1–5 MHz) was used to acquire sonographic measurements of the spleen. Splenic biometry, as

described by Dittrich et al.,²⁶ was carried out in the right-recumbent position, where the organ was measured in three dimensions (L=length, D=depth, B=breadth). Spleen volume was then calculated using the formula for volume determination of an ellipsoid.

$$SV = L * B * \{(D_L + D_B)/2\} * 0.523 [\text{cm}^3]$$

Sonographic measurements were obtained at admission and 24 hours by the study author, SK. Measurements were recorded and processed using standardized pre-set software. All images were digitally recorded in DICOM loops and still frames, and stored for review by a second reviewer, CM.

Spleen volume normogram

As spleen size varies with age, genetic and environmental factors,^{26–29} previously described splenic normograms in other populations were not considered to be suitable controls. Reference values for SV in community controls were estimated based on SV measured in children from the area. Healthy non-hospital based community controls were recruited from three groups. Children aged 3 months to 3 years were recruited from the ‘well baby clinic’ at MRRH. Children aged 4 years and over were recruited from a local child outreach centre and a local day care centre. No clinical data or haematological samples were collected from this population. Children with chronic disease or febrile illness in the preceding two weeks were excluded. Spleen volume was measured using the methods described. Demographic and anthropometric data were collected and SV normograms were constructed for the independent variables: height, weight, total body surface area (TBSA) and age. All data were partially anonymized.

Analysis and statistics

Data were entered on Filemaker Pro (Santa Clara, CA USA) and exported into the Statistical Package for the Social Science (SPSS v19, Chicago, IL, USA) for analysis. Anthropometric data (height, weight and age) from community controls was fit by linear regression, and Pearson correlation coefficients were generated for each regression curve. TBSA was calculated using the methodology described by Mosteller³⁰ and analysed for fit by linear regression. Given that SV data were not normally distributed, values were converted by log_e (*ln*) transformation for analysis. Controls were grouped by TBSA (which had the best regression fit) to generate SV reference values for each group. For comparison of SV across all TBSA groups, standardized z-scores $\{(x-\mu)/\sigma\}$ were calculated based on mean *lnSV* for each TBSA grouping. Significance testing was carried out using analysis of variance (ANOVA) for comparison of means across more than two groups. Student’s t-test with two-tailed significance was used for comparison of two means. χ^2 was used for analysis of proportions, and Fisher’s exact test was used for analysis of proportions in groupings with small sample size. Ninety-five percent confidence intervals (95% CI) were reported for means and p-values are given for each analysis.

Results

Community control data

In total, 241 children were screened as controls. Children with chronic disease and those with a history of febrile illness in the preceding two weeks were excluded. After screening, 186 children met inclusion criteria and were enrolled in the control arm. Fifty-two percent of controls were male and ages ranged from 3 months to 12 years. Pearson correlation coefficients for SV and three anthropometric comparisons plus age are presented in Figure 1 and Table 1. Correlation was best for age (0.760), although reported age was occasionally unreliable in both control and study populations, and hence anthropometric measures were preferred. Of the anthropometric parameters, correlation was best for TBSA (0.723) and thus TBSA was used as the grouping parameter for SV normals. Community controls were split into seven TBSA groupings. Given that SV data were not normally distributed, $\log_e$ transformation of SV was carried out to facilitate parametric analysis. Spleen volume normals are presented in Table 2. The $\ln$ SV means and SDs were then used to calculate SV z-scores for subsequent use in the severe malaria cohort to facilitate intergroup comparison.

Severe malaria population

During the study period, 104 children with severe malaria²³ were enrolled whose median age was 19.2 months (IQR=8.6–33.1). The cohort was comprised of children with cerebral malaria (n=15), severe malarial anaemia (n=54) and other severe malaria (OSM) (n=35). The majority of the children in the OSM group (n=33, 94%) presented with rapid breathing or respiratory distress. Children with coma (CM) who also had a Hb<5 g/dl (n=7) were classified in the CM group. Baseline clinical and laboratory parameters were summarised for each of the three groups (Table 3). Of note, 93% (n=14) of the CM group were males, versus 51% male sex ratio in the remaining groups. Mean Hb was 3.47 g/dl (95% CI 3.21–3.72) in the SMA group, 4.73 g/dl (95% CI 3.58–5.88) in the CM group, and 7.98 (95% CI 7.24–8.73) in the OSM group. Mean lactate was significantly higher in the CM group, 9.81 mmol/L (95% CI 7.45–12.2) vs 6.74 (95% CI 5.44–8.05) in the SMA group. Clinical jaundice was common in the both SMA (n=45, 83.3%) and CM (n=13, 86.7%) groups. Case fatality rate was highest in the CM group, n=4 (26.7%), vs n=1 (1.9%) and n=1 (2.9%) in the SAM and OSM groups respectively.

Spleen volume and disease phenotype

Compared to TBSA-adjusted community controls, SV z-scores in all children with severe malaria were elevated (Table 3). Comparing SMA to CM, children with the SMA phenotype had significantly higher SV, mean z-scores=2.73 (95% CI 2.41–3.04) compared to those with CM, mean z-score=1.98 (95% CI 1.38–2.57) $p=0.028$. For children who died, SV was similar to TBSA-adjusted community control values (mean z-score=1.20 [95% CI 0.14–2.25]), whereas all survivors had elevated SV (mean z-score=2.58 [95% CI 2.35–2.81]); $p=0.004$. Summary data for SV and clinical groupings are presented in Table 4.

Discussion

We presented, for the first time, an ultrasonographically described splenic normogram for children residing in a malaria hyper-holoendemic region of East Africa. We found that TBSA correlated best with SV ($r=0.72$) in healthy controls and therefore used TBSA-adjusted Z-scores for inter-group comparison. The severe malaria cohort included 15 children with CM, 54 with SMA and 35 with OSM including respiratory distress and shock. Median age was similar across the cohort; children with CM were more hypoxaemic and had higher lactate levels compared to other groups. Spleen volumes in children with severe malaria were elevated compared to community controls. Children with SMA had higher SV compared to children with CM. Children who died had similar SV z-scores to that of community controls.

Previous ultrasonographically determined spleen nomograms carried out in Senegal²⁹ and Zimbabwe²⁸ were performed mostly on older children and adults who were non-parasitaemic and resided in *Schistosoma* endemic areas. Given the age differences, comparisons were only possible in a small subset of individuals. For those comparisons, median SV in our control population was significantly higher than those found in Zimbabwe. In the study carried out in Senegal, spleen height was reported instead of SV. In those groups for which comparison was possible, we again found that our spleen height was increased (data not presented). The earliest description of a sonographically defined SV normogram in German children showed similar values to ours for SV in very young children (<70 cm height). However, values quickly diverged and became significantly larger for children over 70 cm in our cohort, which roughly correlated with an age of 9–12 months. Given the high malarial transmission pressures in our region of study, these early increases in SV may be explained by a high exposure to malaria parasites at a very young age and the early development of malarial immunity.

Mortality in the CM cohort was high, 27%, while mortality in the SMA and OSM groups was 2 and 3%, respectively. Sample sizes were relatively small however these rates were similar to other studies carried out in high transmission settings.^{6,31,32} In our study, 14 out of 15 children with CM were males (93%). We could not ascribe a plausible hypothesis for this discordant finding and could only speculate that this was a chance phenomena attributed to the small sample size or possibly related to a pattern of gender biased health seeking behaviour.

In support of our study hypothesis, we found that TBSA-adjusted SV was increased in SMA compared to CM. Although numbers were small, they support the findings of recent studies showing the mechanism and potential impact of splenic function on clinical phenotype.³³ The microcirculation within the spleen involves two pathways: one a fast and closed circulation accounting for 80–90% of the splenic blood flow, the second a slow open circulation involving narrow and short inter-endothelial slits in the sinus wall that RBCs must cross to get back to the general circulation.³⁴ It has been hypothesized, and demonstrated in parasitized red blood cells (PRBCs), that the younger ring forms of PRBCs and less deformable non-parasitized red cells (nPRBC) navigate through the slower open circulation triggering their clearance from the circulation and retention in the spleen.³⁵ This

may lead to a substantive increase in the SV, which we witness in severe malaria, but is exaggerated in children with SMA, which in turn results in red cell destruction of PRBCs and nPRBCs. This in turn reduces the speed of parasite-load increase and therefore the pace at which infection proceeds. The aetiology of acute SMA involves red cell loss, with greater contribution of nPRBCs than PRBCs. Our findings, that children with SMA have a larger splenic volume, could be attributed to the greater clearance and retention of young rings and poorly deformable nPRBCs, with resultant severe anemia. Recent studies using histidine rich protein-2 to define parasite load in severe malaria further support the hypothesis that cerebral malaria and fatal malaria may be triggered by cells which escape splenic removal, as parasite factors resulting in a larger parasite load and sequestration correlate both with severity, cerebral malaria presentation and fatal outcome.^{36–38}

While study numbers were small, the absence of SV increase in fatal cases (Table 4), suggests that splenic enlargement may be protective and may be related to a heightened splenic immune function, which sequesters infected RBCs and provides an immune advantage through decreased circulating parasite biomass. In previous studies where splenomegaly was assessed by clinical examination, the association between clinical splenomegaly and mortality has been conflicting. A study from Ifakara Tanzania, where the EIR was similarly over 300, found that splenomegaly was associated with an increased risk of malarial death in young children (OR 2.9, 95% CI 1.0–8.9).³² In contrast, a study in a meso-endemic setting in Mali, found a hazard ratio of 0.33 (95% CI 0.15–0.72) with splenic enlargement being protective from mortality.³⁹ Finally, a Gambian study demonstrated no association between clinical splenomegaly on presentation and mortality (OR 0.6, 95% CI 0.4–1.0), although the trend was towards a protective function of splenic enlargement.³¹ Our data from a hyperendemic region suggest that an increase in spleen volume during an episode of severe malaria may be protective against CM and mortality and that the absence of acute splenic enlargement in acute *P. falciparum* malaria may be associated with increased mortality. These conclusions remain speculative, although taken together with epidemiologic observations of protective hypersplenism in immune populations^{32,39} our findings suggest that splenic enlargement may have a role in protecting against CM and death in high transmission settings. These conclusions need to be established in prospective studies.

Conclusion

In this study, we demonstrated that SV was increased in comparison to TBSA matched control values from the community in cases of acute *P. falciparum* malaria. Furthermore, we showed that mean TBSA-adjusted SV z-scores were greater in children with SMA compared to CM. We also found that in children who died from severe malaria, there was no increase in SV compared to healthy community controls. We speculate that splenic enlargement may protect from CM disease phenotype and may afford some degree of clinical immunity. Future studies should characterize the immunologic properties of hypersplenism as a corollary to malaria clinical immunity.

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Figure 1.

Linear regression curves for $\ln$ spleen volume against anthropometric parameters and age for community controls. Best-fit lines (solid) and associated 95% CIs for expected mean values (dashed). TBSA: total body surface area (m^2).

Table 1

Pearson correlation coefficients for linear regression analysis of anthropometric measure against spleen volume

Metric	All	Male	Female
	n=186	n=97	n=89
Height (cm)	0.707	0.674	0.733
Weight (Kg)	0.716	0.695	0.705
TBSA (m ²)	0.723	0.697	0.727
Age (months)	0.760	0.738	0.762

TBSA: total body surface area.

Table 2

Spleen volume reference values grouped by total body surface area (TBSA) in healthy controls

TBSA (m ²)	n		Spleen volume (ml)		(ln) Spleen volume
<0.39	15	Median	26.0	mean	3.21
		IQR	(21.5–34.0)	SD	0.31
0.40–0.54	15	Median	36.5	mean	3.63
		IQR	(27.8–47.8)	SD	0.34
0.55–0.69	23	Median	67.0	mean	4.17
		IQR	(54.0–88.0)	SD	0.34
0.70–0.84	50	Median	110	mean	4.75
		IQR	(89.5–149)	SD	0.45
0.85–0.99	36	Median	161	mean	5.09
		IQR	(108–256)	SD	0.52
1.00–1.14	31	Median	267	mean	5.49
		IQR	(182–355)	SD	0.46
1.15+	16	Median	249	mean	5.58
		IQR	(190–401)	SD	0.38

Medians and IQRs for true volumes (ml).

ln Spleen volume (natural log of spleen volume) mean and SD.

Table 3

Clinical and laboratory features of severe malaria cases, grouped by disease type

	All n=104 Median (IQR)	OSM n=35 Median (IQR)	SMA n=54 Median (IQR)	CM n=15 Median (IQR)	p-value
Age (months)	19.2 (8.6–33.1)	21.5 (9.6–36.0)	15.9 (8.5–30.9)	19.8 (7.9–35.1)	NS ^a
	Mean (95%CI)	Mean (95%CI)	Mean (95%CI)	Mean (95%CI)	ANOVA
Respiratory rate (brpm)	57.6 (55.4–59.7)	53.1 (49.5–56.7)	60.3 (57.2–63.4)	58.3 (54.0–62.6)	0.010
Heart rate (bpm)	167 (162–172)	176 (169–184)	165 (158–173)	152 (134–170)	0.009
Axillary temp (°C)	37.8 (37.6–38.1)	38.5 (38.2–38.9)	37.5 (37.2–37.7)	37.2 (36.1–38.4)	<0.001
MAP (mmHg)	68.9 (65.7–72.2)	73.3 (70.2–76.5)	66.8 (61.6–72.0)	66.7 (56.4–77.0)	NS
Oxygen saturation (%)	90.9 (88.8–93.0)	93.0 (91.3–94.6)	92.8 (90.9–94.7)	79.2 (68.0–90.4)	<0.001
Length of stay (days)	2.46 (2.20–2.72)	2.37 (1.99–2.75)	2.33 (2.05–2.62)	3.13 (1.78–4.49)	NS
Glucose (mg/dl)	7.69 (7.14–8.23)	7.04 (6.37–7.71)	8.25 (7.51–8.99)	7.16 (4.82–9.50)	NS
Hemoglobin (g/dl)	5.17 (4.66–5.68)	7.98 (7.24–8.73)	3.47 (3.21–3.72)	4.73 (3.58–5.88)	<0.001
Lactate (mmol/L)	6.27 (5.36–7.18)	4.00 (2.84–5.17)	6.74 (5.44–8.05)	9.81 (7.45–12.2)	<0.001
SV z-score	2.50 (2.27–2.73)	2.38 (1.98–2.78)	2.73 (2.27–2.73)	1.98 (1.38–2.57)	NS
	n (%)	n (%)	n (%)	n (%)	χ^2
Sex (% male)	59 (56.7)	18 (51.4)	27 (50)	14 (93.3)	0.008
Hepatomegaly	34 (32.7)	8 (22.9)	18 (33.3)	8 (53.3)	NS
Jaundice	68 (65.4)	10 (28.6)	45 (83.3)	13 (86.7)	<0.001
Mortality	6 (5.8)	1 (2.9)	1 (1.9)	4 (26.7)	0.001

bpm: beats per minute; brpm: breaths per minute; CM: cerebral malaria; MAP: mean arterial pressure; NS: not significant; OSM: other severe malaria; SMA: severe malarial anemia; SV: spleen volume.

^aKruskal–Wallace.

Table 4

Spleen volume (SV) z-scores in cases of severe *Plasmodium falciparum* malaria for given clinical parameter

SV z-score – mean (95% CI)		p-value
Male (n=59) 2.67 (95% CI 2.37–2.95)	Female (n=45) 2.36 (95% CI 1.98–2.74)	NS
Alive (n=98) 2.58 (95% CI 2.35–2.81)	Died (n=6) 1.20 (95% CI 0.14–2.25)	0.004
No SMA (n=43) 2.40 (95% CI 2.05–2.76)	SMA (n=61) 2.61 (95% CI 2.31–2.92)	NS
No CM (n=89) 2.62 (95% CI 2.38–2.87)	CM (n=15) 1.94 (95% CI 1.30–2.58)	NS
SMA (n=54) 2.73 (95% CI 2.41–3.04)	CM (n=15) 1.98 (95% CI 1.38–2.57)	0.028
No clinical jaundice (n=34) 2.54 (95% CI 2.13–2.95)	Clinical jaundice (n=67) 2.52 (95% CI 2.24–2.81)	NS
Lactate <5 mmol/L (n=48) 2.34 (95% CI 1.98–2.69)	Lactate ≥ 5 mmol/L (n=53) 2.71 (95% CI 2.40–2.73)	NS

Comparison of mean SV z-scores for clinical groupings using total body surface area (TBSA) matched community controls as reference values.
CM: cerebral malaria; NS: not significant; SMA: severe malarial anemia.