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HEMOGLOBIN CONCENTRATION IN CHILDREN IN A MALARIA HOLOENDEMIC AREA IS DETERMINED BY CUMULATED *PLASMODIUM FALCIPARUM* PARASITE DENSITIES

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Abstract. In malaria holoendemic areas children are anemic, but the exact influence of falciparum malaria on hemoglobin (Hb) concentration remains largely unsettled. Prospective data were therefore collected in children < 24 months of age during five months in a Tanzanian village. Children with mean asymptomatic parasitemia $\geq 400/\mu l$ had lower median Hb levels during the study than those with mean density $< 400/\mu l$. The difference was 9.7 g/L (95% confidence interval [CI] 2.8–17). In children with one or more clinical malaria episodes, the median Hb was 8.3 g/L (95% CI 0.9–16) lower than those without episode. If early treatment failure was recorded, the immediate effect on Hb was particularly important with a mean drop of 17 g/L. Interestingly, at study-end the Hb concentration represented a function of the area under the parasitemia curve (AUPC) during the previous five months, adjusting for age. In conclusion, stepwise deterioration in median Hb levels was found by asymptomatic parasitemia, clinical malaria episode, and most significantly, treatment failure.

INTRODUCTION

In sub-Saharan Africa, morbidity and mortality from childhood anemia are severe public health problems.[1] The main contributory causes of anemia are infectious diseases, nutritional disorders, and hemoglobinopathies.[2] Some investigators cite an iron-deficient diet, hookworm infection, or schistosomiasis as important causes for anemia,[3–5] while others have identified malaria as the primary cause. Cross-sectional data have shown deterioration in hemoglobin (Hb) concentration during malaria transmission and an improvement in Hb with the administration of malaria chemoprophylaxis or insecticide-treated bed nets.[6–8] These studies do not fully exclude other conditions associated with malaria, but *Plasmodium falciparum* infection appears to be a major factor in Hb change.[9]

During infancy, acute malaria episodes have consistently been associated with low hematocrit < 25%,[10] mainly occurring from four months of age when children are at risk of clinical attacks.[11,12] However, malaria-associated anemia may also develop without fever, and be undiagnosed as clinical malaria requiring treatment.[13] It has therefore been suggested that children aged < 36 months living in an area with *P. falciparum* malaria should be routinely treated for malaria if they have signs of anemia.[14]

The causal relationship between *P. falciparum* parasitemia and Hb concentration in malaria-holoendemic areas is difficult to establish since most children continually harbor parasites.[15] Furthermore, the impact on Hb of clinical episodes and asymptomatic long-term parasitemia may be difficult to distinguish.[16] Cross-sectional data or data from hospitalized children with severe anemia are unable to capture the dynamic relationship between malaria and anemia,[17–19] and only longitudinal data may reveal whether a low Hb level is the result of recent high parasite density or of longstanding parasitemia.[20] Furthermore, the pathophysiologic processes of anemia in malaria include both hemolytic destruction of erythrocytes and bone marrow suppression,[21] implying different time-lags between parasitemia and subsequent effects on Hb levels,[22] and hence the need for prospective data.

The objective of the present study was to prospectively investigate the impact of symptomatic and asymptomatic parasitemias on Hb levels in children living in a rural area with holoendemic malaria.

PATIENTS AND METHODS

Study design. Data for the present study were collected from a prospective cohort of 211 Tanzanian children participating in a randomized controlled intervention trial documenting the effects of regular micronutrient supplementation on malaria and anemia.[23] Descriptive data of the whole cohort are presented below, but analyses have been restricted to the 103 subjects from the placebo group.

The study was conducted from June to November 1995 in Fukayosi village, 30 km west of Bagamoyo town, coastal Tanzania. Malaria is holoendemic and transmission is perennial with a seasonal peak in July. A census of all children aged between five and 36 months was performed by members of the village committee, and 211 out of 220 children enumerated were enrolled. No child had to be excluded due to congenital malformations, clinically decompensated anemia, or migration plans, but four subjects did not participate in the randomized controlled trial due to having an initial Hb concentration < 50 g/L. Informed consent was obtained from all parents for 20 weeks' follow-up and randomization of the intervention. On enrollment, all children received an identity number and a ''Mother-and-Child-Health'' card. Demographic data, including age, were obtained from the card held by each child's parent, and the approximate distance from their home to the village dispensary was estimated by fieldworkers after the start of the study. A medical examination, including weight, spleen size, and axillary temperature, was made by one of the investigators and capillary blood was collected for thick blood films and hematological measurement. Any history of traditional uvulectomy was elicited as this could affect Hb concentration.

The children were seen every second week by the research team at the village dispensary for medical examination and axillary temperature measurement. These 11 dispensary vis-

its, including baseline and end surveys, constituted the active case detection. In addition, six team workers made home visits three times a week which equaled 56 occasions per child for supervised administration of placebo or supplement. Any child with a febrile history would then be referred to the dispensary ("actively passive" case detection). Finally, for passive case detection of fever episodes, the village dispensary was staffed around-the-clock by an assistant doctor and nurse from the team, and daily by at least one of the principal investigators. Study participation was recorded both at the bi-weekly dispensary examinations and during the regular home visits on alternate days. Activities of all team members were continually supervised by the principal investigators.

Clinical malaria episodes. A standardized medical examination was performed in all children with an axillary temperature $\geq 37.5°C$. This included symptoms and signs of diseases other than malaria, as well as an estimation of the clinical severity of the current episode. Capillary blood was collected for immediate preparation and examination of a thick blood film, with an additional 40 μl pre-diluted for hematological measurements including Hb concentration. The pre-diluted specimens were transported by car in a cool-box for approximately one hr before being analyzed. Results were available at the dispensary the next day. Fever episodes judged to be non-malarial in origin were treated accordingly; co-trimoxazole was not used as an antibiotic due to its antimalarial effect. After clinically excluding other febrile conditions, a *P. falciparum* parasite density > 4,000 asexual parasites per μl, estimated during the immediate slide examination in the field was considered indicative of a clinical malaria episode requiring treatment.

Malaria episodes were treated with chloroquine syrup (Elyquine® by Elys Ltd, Dar es Salaam, Tanzania) in accordance with Ministry of Health guidelines and administered by a team nurse. Ten mg/kg of chloroquine base was given on Day 0, followed by 10 mg/kg on Day 1, and five mg/kg on Day 2. Every intake was supervised and children were subsequently observed at the dispensary for 20 min. In case of vomiting, a full dose was repeated and the child observed as before. Axillary temperature was measured daily until Day 3.

Routinely, capillary blood was collected again on Day 3 for thick blood films and hematologic measurement. In case of persistent fever on Day 2, or clinical deterioration before Day 3, the child was re-evaluated and blood collected for hematologic and immediate parasitologic examination; children with early treatment failure were given sulfadoxine-pyrimethamine (SP) and referred to the district hospital, if necessary. All in-hospital drugs for study children were provided by the team. Late clinical treatment failures with fever and parasitemia reappearing between Days 4–13 were treated with SP and paracetamol. Recurrence of clinical malaria episodes from Day 14 were assumed to be due to re-infection and treated as new episodes. All new resumptions of fever during the 20 weeks' follow-up were recorded, classified, and treated as described.

Classification of therapeutic response. Therapeutic response within the first 14 days of treatment was defined according to World Health Organization (WHO) guidelines.[24] However, since subjects' adherence to the study protocol did not allow capillary blood collection frequently enough, a modification was made. Early treatment failure (ETF) was defined according to the WHO (1996) document, but late treatment failure (LTF) or adequate clinical response (ACR) was based on blood and clinical examinations performed two to four weeks after treatment at the regular dispensary visit on or next after Day 14.

Early treatment failure was defined by the following criteria:

- Danger signs (unconsciousness, prostration, convulsion, excessive vomiting, or no oral intake) within 72 hr, and asexual *P. falciparum* parasitemia.
- Temperature $\geq 37.5°C$ after 48 hr, with asexual parasitemia > Day 0.
- Temperature $\geq 37.5°C$ after 72 hr with asexual parasitemia.
- Asexual parasitemia after 72 hr $\geq 25\%$ of Day 0.

Late treatment failure was defined as:

- Danger signs and asexual parasitemia during Days 4–14, without meeting the criteria for ETF.
- Temperature $\geq 37.5°C$ and asexual parasitemia during Days 4–14, without meeting the criteria for ETF.

Adequate clinical response was defined as:

- Temperature < $37.5°C$ with asexual parasitemia during Days 15–28, without meeting the criteria for ETF or LTF.
- No asexual parasitemia during Days 15–28, without meeting the criteria for ETF or LTF.

Scheduled blood collections. Blood samples were drawn from all children at the baseline and end surveys and in two surveys after approximately two and four months. Capillary blood was also collected twice during each clinical malaria episode and once at follow-up. All collections included a blood slide and Hb estimation.

Laboratory tests and quality control. Thick blood films were stained with 5% Giemsa stain, and the asexual parasite density was estimated by counting parasites against 200 white blood cells (WBC), assuming a standard WBC count of $8,000/μl$.[25] If less than 10 parasites were recorded per 200 leucocytes, estimation was made against another 300 WBCs. All *P. falciparum* parasitemias, whether single or dual infections, were used in the analyses. The slides of febrile patients were first examined in the field. All slides were later read by the investigator who examined all blood slides, with 10% re-checked by two blinded, independent microscopists. The between-reader variability of parasite density was < 1.5 log.

For hematologic measurements, an automated hematologic analyser (Cell-Dyn® 610, Abbot Laboratories) was used. Daily commercial quality controls assessed the coefficient of variation for the within-sample precision of the Hb estimate at < 1%, both for immediate run samples and after a six-hour delay in analysis. Capillary blood collection was standardized to prevent pre-analytic bias in Hb estimation. All fingerprick specimens were collected during morning hours, if possible. The children were sitting upright in their mothers' laps, allowing free flow of capillary blood. Two team members performed all blood collections.

Screening was performed for conditions potentially af-

TABLE 1
Descriptive data from baseline and end surveys by age

Age group*	Survey	Mean Hb g/L (SD)	Hb < 70 g/L No. (%)	Parasitemic children No. (%)	Parasite density/μl geometric mean of all slides
< 24 months	Baseline	80 (14)	16/46 (35%)	43/46 (94%)	3,100
< 24 months	End	88 (14)	6/43 (14%)	35/43 (81%)	670
≥ 24 months	Baseline	97 (14)	1/57 (2%)	50/57 (88%)	920
≥ 24 months	End	96 (13)	2/54 (4%)	49/54 (91%)	1,000
All	Baseline	89 (16)	17/103 (16%)	93/103 (90%)	1,620
All	End	92 (14)	8/97 (8%)	84/97 (86%)	840

* Age at study-end. Hb = hemoglobin; SD = standard deviation.

fecting anemia, but no children had to be excluded from the analyses. Sickle cell hemoglobin was assessed by a solubility test and confirmed by Hb electrophoresis. Human immuno-deficiency virus (HIV) was investigated by enzyme-linked immunosorbent assay (ELISA) (Behring) and confirmed by Western blot. The HIV results were only available after study termination and were blinded; hence, no pre-test counseling was performed. Helminthic infections were investigated but not quantified in a single stool specimen by the formalin-ether concentration technique.[26]

Statistical methods. The data were analyzed using Epi-Info® 6.2 and JMP® 3.1 (SAS Institute Inc.). Clinical malaria incidence rates were calculated based on person-time attendance during home visits. Proportions were compared with the aid of Pearson's chi-square or Fisher's exact test. Paired or two-sample t-tests and analysis of variance were used for normally distributed continuous data; parasite densities were normalized after adding one to each count, using logarithmic transformation. The Wilcoxon rank-sum test was used for non-normal continuous data. A statistical significance level of 0.05, two-tailed test, was generally employed.

Ethical considerations. All work was performed in accordance with the guidelines for human experimentation in clinical research stated in the research approval from the ethical committees of the Karolinska Institute in Stockholm and the Muhimbili College of Health Sciences in Dar es Salaam, Tanzania. Parents of study subjects provided oral informed consent.

RESULTS

Full cohort descriptives. *Study subjects.* The 211 children of the full study cohort had a mean age of 19 months (SD 8.5) at enrollment; 51% were male. The frequency of home-visit attendance revealed that 88% of the children's total person-time was observed and recorded in the study. Study participation was evenly distributed over the 20 study weeks and unaffected by distance from home to dispensary (data not shown). The median attendance at the regular bi-weekly dispensary examinations was 100%, with 190 children (90%) present at ≥ 8 of the 11 scheduled visits. The 45 children living furthest away from the dispensary had a slightly lower attendance record with a median of 91% (interquartile range [IQR] 73–100).

Biochemical and clinical observations. Blood was collected on 1,540 occasions in the 211 study subjects, corresponding to a median of seven observations per child (range 1–19). A median of four (IQR 3–4) blood collections were

made in children with no clinical malaria episodes recorded during the study. There was no fever or ongoing drug treatment at the time of 856 (56%) blood collections. Out of all blood slides, 1,092 (73%) were single infections with *P. falciparum,* 183 (12%) were dual infections that included *P. falciparum,* and 14 (1%) were single infections with another species. No child remained aparasitemic over the full study period.

Out of 388 fever episodes recorded during the five study months, 284 were diagnosed and treated as malaria episodes. Forty percent of fevers were detected at the scheduled bi-weekly visits and 60% upon reporting to the dispensary. A total of 103 (36%) malaria cases were diagnosed in the first month; study onset coincided with peak malaria transmission at the end of the rainy season. The incidence of clinical malaria episodes subsequently dropped and remained constant over the subsequent four months.

During the study period, three children were referred to hospital due to gastroenteritis and malaria, and one of them received a blood transfusion. The children were continually followed during and after hospital admission. The prevalence of the sickle-cell trait was 23%. No child was found positive for HIV infection. Hookworm infection was detected in 13% of the children, but there was no statistical association with Hb concentration, adjusting for age (data not shown).

Placebo group analyses. The following analyses on the relationship between parasitemia and Hb levels are based on data from the 103 children of the placebo group who did not receive micronutrient supplementation.

Age dependence. Data from the cross-sectional surveys at the beginning and end of the study are displayed in Table 1. Children aged < 24 months at study-end had initially significantly lower Hb and more often severe anemia, i.e., Hb < 70 g/L, than older children ($P < 0.001$), but this difference was less apparent at the end of study and not statistically significant for the prevalence of severe anemia ($P = 0.11$). There was also a statistically significant reduction in parasite prevalence and mean density in children < 24 months over the five-month study period (Table 1). The longitudinal data presented in Table 2 revealed a marked age dependence of malaria indices, such as the clinical malaria incidence rate and parasite density at treatment onset. Early chloroquine treatment failure rate was also highest in the youngest children.

Clinical malaria episodes. The parasite density on Day 0 of first or only clinical malaria episode was linearly related to the subsequent drop in Hb after 72 hr ($P < 0.001$; data

TABLE 2
Clinical falciparum malaria episodes, treatment responses to chloroquine, and asymptomatic parasitemias by age

	Age at study-end			
	<18 months	18–23 months	≥ 24 months	All
No. of children at baseline	20	26	57	103
Children with at least one clinical malaria episode recorded, no. (%)	14 (70%)	21 (81%)	28 (49%)	63 (61%)
Mean incidence rate of clinical malaria episodes/person-month (95% CI)	0.38 (0.24–0.52)	0.29 (0.19–0.39)	0.16 (0.11–0.22)	0.24 (0.19–0.29)
Parasite density/µl at start of antimalarial treatment, geometric mean (IQR) of all episodes	25,000 (7,800–70,000)	11,000 (5,000–39,000)	9,000 (4,500–25,000)	13,000 (5,000–41,000)
Children with early treatment failure, no. (%)	8 (40%)	3 (12%)	3 (5%)	14 (14%)
Children with late treatment failure, no. (%)	4 (20%)	5 (19%)	8 (14%)	17 (17%)
Asymptomatic parasite density/µl, geometric mean (IQR)	610 (400–5,000)	1,000 (530–6,300)	740 (600–5,000)	790 (500–5,000)

IQR = interquartile range.

not shown). Early failure of chloroquine treatment was associated with an immediate reduction of Hb concentration within 72 hr, but this was only evident in the presence of hyperparasitemia (Table 3). The magnitude of the fall was unrelated to age, after adjusting for parasite density. Treatment failure was not associated with hyperparasitemia *per se* (data not shown). In 33 of 46 children < 24 months the criteria for chloroquine treatment failure was not fulfilled at any time during the study. In these children, an increasing incidence of clinical malaria episodes was associated with a linear decline in individual median Hb, from 88 to 64 g/L ($P = 0.03$; Figure 1). The interactive effects of clinical malaria episodes, mean asymptomatic parasitemias, and number of negative blood-slides on individual median Hb levels are presented in Figure 2. In children ≥ 24 months, hemoglobin levels were only marginally affected by clinical episodes and parasite densities (Figure 2B). In contrast, in children < 24 months the effect was significant (Figure 2A), and those with at least one clinical malaria episode had 8.3 g/L (95% CI, 0.9–16.0) lower median Hb than those without any episode.

Asymptomatic parasitemia. As seen in Table 2, the overall geometric mean parasite density during asymptomatic intervals and occurrence of negative blood-slides were not associated with age. This relationship was confounded, however, by SP treatment. Sulfadoxine-pyrimethamine treatment, mainly given to the youngest children, resulted in lower parasite density and prevalence in subsequent asymptomatic follow-up specimens; the geometric mean parasitemia in children treated with SP at any time during the study was 200 parasites/µl compared to 1,150 in children without such treatment ($P = 0.003$). As shown in Figure 2A, children < 24 months with asymptomatic mean parasite density ≥ 400/

µl had 9.7 g/L (95% CI 2.8–17.0) lower median Hb levels than those with mean density < 400/µl. Similarly, children who were constantly parasitemic had 10.0 g/L (95% CI 3.3–17.0) lower median Hb than those with > 2 negative blood-slides during the five-month study period.

Malaria indices and Hb. When combined, the malaria indices markedly affected individual median Hb in children < 24 months (Table 4). For example, children with no clinical malaria episode and a geometric mean asymptomatic parasite density < 400/µl had a median Hb of 104 g/L. This level decreased to 85 g/L if at least one clinical malaria episode occurred and to 83 g/L if the mean asymptomatic parasitemia increased to ≥ 400/µl. In case of both, the median Hb fell to 79 g/L. To reach a median Hb concentration > 100 g/L, children < 24 months in this study had to have no clinical malaria episode, a mean parasitemia < 25 parasites/µl, and at least two negative blood-slides. No cohort child < 24 months had Hb > 110 g/L consistently, the Centers for Disease Control and Prevention (CDC) criterion for a normal Hb level.[27]

Cumulated parasitemia. The association between simultaneous parasite density and Hb was inconsistent; there was an association in the baseline survey, but not in the end survey, nor in each subject's first or only clinical malaria episode (data not shown). Similarly, the geometric mean symptomatic parasitemia and the occurrence of symptomatic parasitemias > 20,000/µl did not influence the individual median Hb during the study period, even after adjusting for age and SP treatment. Furthermore, there was no association between baseline Hb and parasitemia in the first malaria episode, but baseline parasitemia was significantly related to Hb in the first malaria episode. To allow for a longer influ-

TABLE 3
Change in hemoglobin (Hb) (g/L) between Day 0 and Day 3 in each subject's first or only clinical falciparum malaria episode

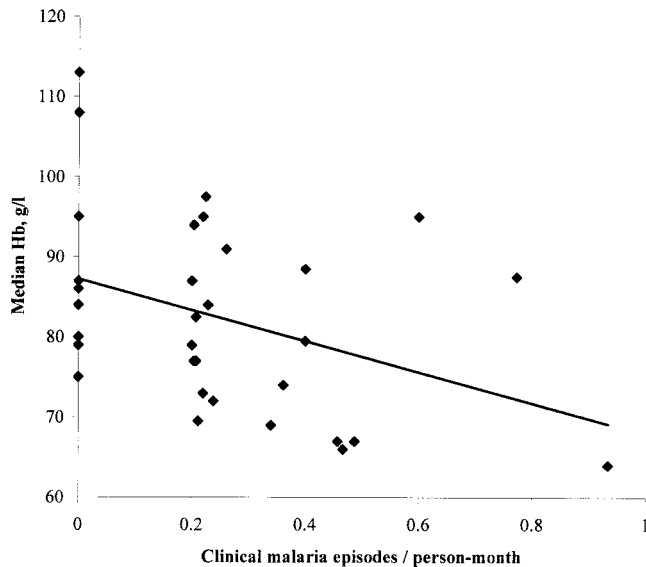|	Change in Hb 72 hours after starting chloroquine treatment, g/L (SE)		
Chloroquine treatment responses	Parasitemia < 20,000/µl on Day 0	Parasitemia ≥ 20,000/µl on Day 0	All
Early treatment failures	+1 (3), n = 4	−17 (3), n = 6	−10 (4), n = 10
Late treatment failure + adequate clinical responses	−2 (1), n = 25	−3 (2), n = 13	−2 (1), n = 38

n = number of patients.

FIGURE 1.   Association between incidence of clinical malaria episodes and individual median Hb in children aged < 24 months not fulfilling the criteria for chloroquine treatment failure and not receiving treatment with sulfadoxine-pyrimethamine (SP). Correlation coefficient, r = 0.38; P = 0.03.

ence of parasitemia, each individual's parasite load over five months was estimated as the area under the parasitemia curve (AUPC) by multiplying the geometric mean of two consecutive parasite densities with number of days between measurements (Figure 3). Adjusting for age, the AUPC was highly significant in explaining the final Hb concentration. Using only asymptomatic, symptomatic, or cross-sectional parasitemia recordings produced a less satisfactory fit of the regression model.

Severe anemia. Severe anemia, defined as median Hb < 70 g/L, was recorded in 7 of 46 children aged < 24 months despite optimal case management using local treatment guidelines. Children with severe anemia were unlikely to have had any negative blood-slides during the study, relative risk (RR) 0.3 (95% CI 0.09–1.0). They had higher mean asymptomatic parasite density (2,600/μl) than children without severe anemia (730/μl; P = 0.01), and their mean clinical malaria incidence rate was 0.56/person-month compared to 0.29 (P = 0.01).

Within-individual Hb fluctuations. The mean Hb improvement from study start to end in children without clinical malaria episodes was 2.1 g/L in subjects < 24 months and 1.2 g/L in those ≥ 24 months. The mean variation in individual Hb estimates during the study was 14 g/L and 12 g/L, respectively. Hence, in addition to a net increase in Hb, there was substantial individual fluctuation in the repeated Hb estimates. Variation in Hb due to changes in parasitemia would presumably occur. The geometric mean within-individual variation in parasitemia in the same asymptomatic individuals was 4,200 (IQR 2,200–9,300) parasites/μl; this variation was, however, unrelated to fluctuations in Hb (P = 0.60). In contrast, the fluctuations in Hb were significantly related to episodes of both vomiting and diarrhea (data not shown).

DISCUSSION

To determine the influence of malaria parasitemia on Hb concentration, children in the present study were followed prospectively for five months in a clinical setting at village level under optimal local diagnostic and treatment conditions. Although mostly mild and moderate malaria cases abound at community level, such cases may precede severe disease manifestations, including severe anemia, that are seen in children admitted to hospital. Furthermore, it is probably more appropriate to study the basic interaction between parasite and host at community level since this interaction is less distorted by complicating factors, including antimalarial treatment before the onset of severe symptoms requiring hospitalization. In addition, a longitudinal perspective was considered essential when trying to capture a pathogenetic time-lag of the effects of parasitemia on Hb.

Capillary Hb estimates in children are prone to pre-analytic variation and bias.[28] In the present study, dehydration due to vomiting and diarrhea was associated with within-individual fluctuations in Hb concentration. In addition, technical sampling bias may be a problem in children who are sick and less cooperative during capillary blood collection. To reduce the effects of methodological variability in repeated capillary Hb estimates, the median of an individual's Hb readings over five months was used. This value, reflecting the individual's long-term Hb-profile, was correlated with clinical and parasitological data collected during the study period. The overall five-month observation time extended well beyond the expected regeneration time of red blood cells, allowing for an Hb 'steady state' level to be reached under the present diagnostic and treatment conditions.

The differences in parasitemia and Hb between the baseline and end surveys probably reflect the intensified malaria case management during the study. The laboratory indices of children ≥ 24 months were unchanged from study start to end, whereas those of children < 24 months showed only modest improvement (Table 1). The continued high prevalence of parasitemia and low mean Hb levels at the end survey represent the end results of five months of prompt malaria case management in a holoendemic area. Whether the use of sulfadoxine-pyrimethamine instead of chloroquine as first-line treatment would have improved these results remains undetermined. However, the median Hb during the study remained unaffected by SP treatment, emphasizing its temporary impact on Hb levels. This, therefore, demonstrates the limitations of 'case management' as the only malaria control tool, using current treatment guidelines.

There was an excessive vulnerability to symptomatic malaria in the youngest children (Table 2). In addition, 40% of children < 18 months were affected by early chloroquine treatment failure during the five months' observation period, leading to a concomitant drop in Hb, especially following hyperparasitemia (Table 3).[29] Hence, it was predominantly the youngest children who suffered attacks of severe acute hemolytic anemia. All treatment failures received prompt second-line therapy with SP, which at follow-up compensated for the immediate loss in Hb, most likely by subsequent reduction in the asymptomatic parasitemia level and possibly clinical malaria incidence rate. Since the availability of ef-
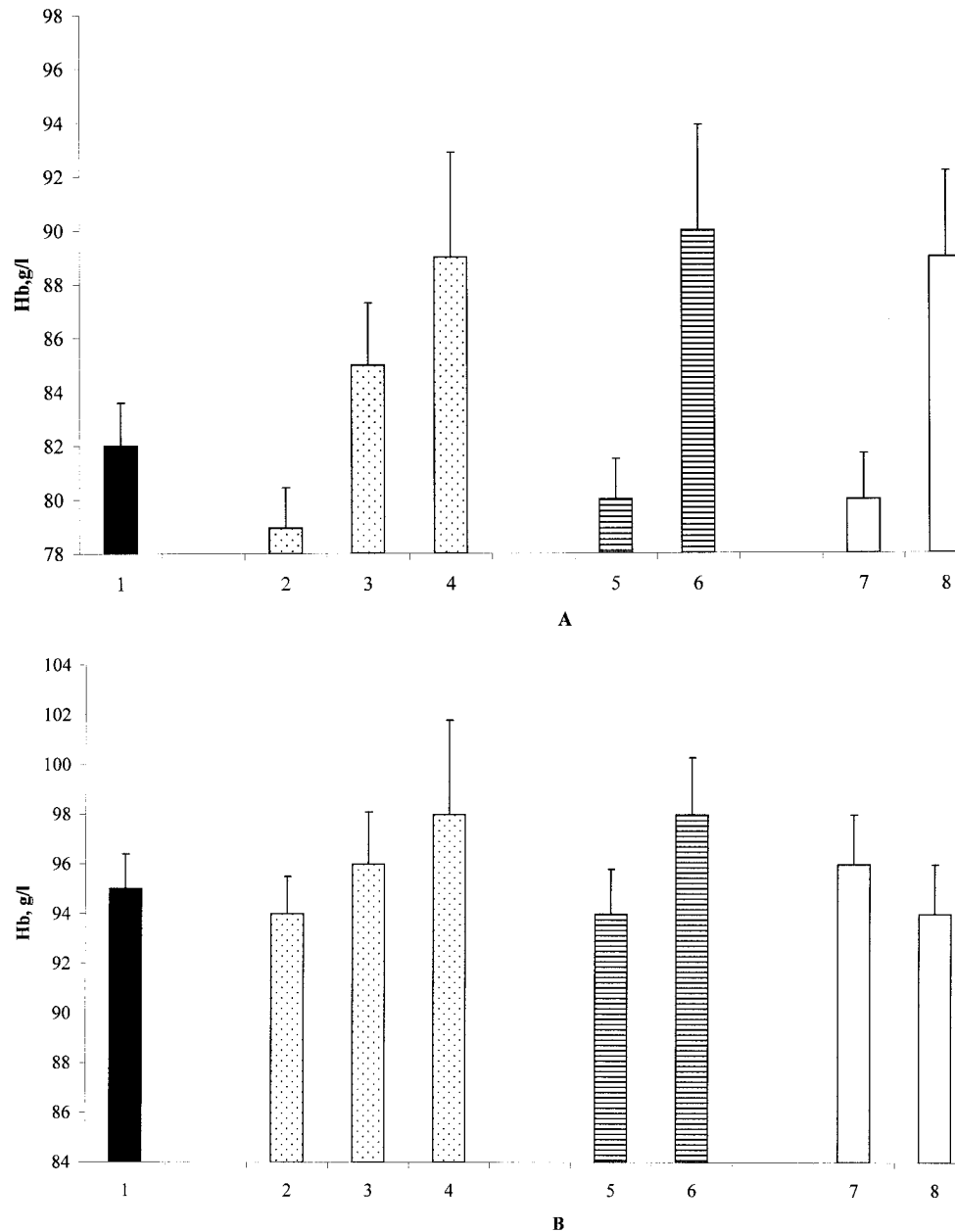
FIGURE 2.   Effects of malaria parasitemia and clinical malaria episodes on mean of individual median Hb, g/L (SE). Figure A represents children aged < 24 months at study-end and Figure B, children ≥ 24 months.

Column 1 = overall mean Hb
Column 2 = no negative blood-slide
Column 3 = ≥ 1 negative blood-slide
Column 4 = > 2 negative blood-slides
Column 5 = geometric mean asymptomatic parasitemia ≥ 400 parasites/μl
Column 6 = geometric mean asymptomatic parasitemia < 400 parasites/μl
Column 7 = ≥ 1 clinical malaria episode
Column 8 = no clinical malaria episodes

fective second-line antimalarial therapy at village level may be inconsistent under non-study conditions, it is likely that acute anemia due to early chloroquine treatment failure is a major preventable contributor of anemia currently observed in young children. Furthermore, chloroquine treatment, even without therapeutic failure, could not prevent a general reduction in Hb levels (Figure 1). It was not possible to determine whether this was due to cumulated recurrent loss of

Hb during the repeated malaria episodes *per se,* or to a higher post-treatment asymptomatic parasitemia level compared to SP.

There was a clear dose-response relationship between *P. falciparum* parasitemia and Hb in children younger than two years (Figure 2A). In addition, the Hb concentration at study-end was found to be a cumulative function of the previous parasitemias over five months, adjusting for age. In

TABLE 4
Combined effects of malaria indices on mean of individual median hemoglobin (Hb) g/L (SE) in children < 24 months old

	No. clinical malaria episodes		No. negative blood-slides	
	None	≥1	≥1	None
No. clinical malaria episodes				
None			104 (7.0), n = 10	83 (2.2), n = 30
≥ 1			82 (2.0), n = 38	78 (2.6), n = 25
Asymptomatic parasite density				
< 400 mm³	104 (7.0), n = 8	85 (3.2), n = 18	90 (3.9), n = 26	
≥ 400 mm³	83 (2.2), n = 31	79 (1.8), n = 44	80 (2.6), n = 22	80 (1.9), n = 53

SE = standard error.
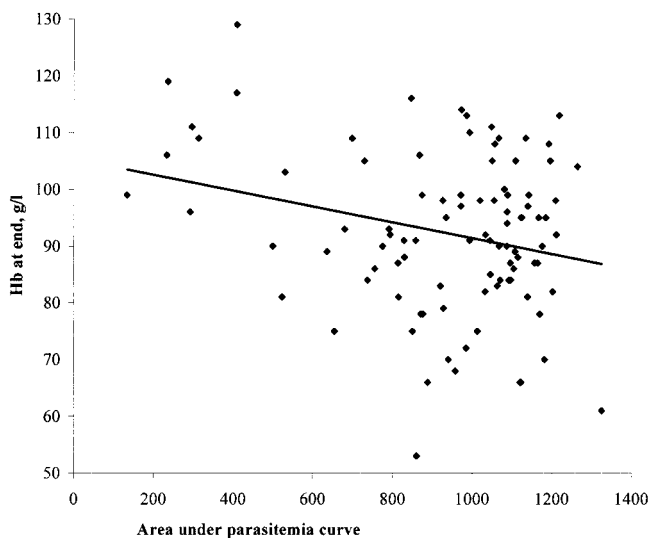n = number of patients.

the formula for AUPC each symptomatic parasitemia was given a weight of three days, out of a total study duration of 140 days. The extension of duration of symptomatic parasitemias was prevented by immediate drug intervention in the case of treatment failure. In addition, logarithmic transformation of parasite counts further reduced the relative importance of high symptomatic parasite densities. Hence, in the sampling scheme used, symptomatic parasitemias were restricted both in time and magnitude compared to asymptomatic parasitemias. Considering the excellent model fit under this scheme, it seems plausible that asymptomatic para-

Analysis of variance	Df	Sum of squares	F-ratio	Prob>F
Model	2	3,250	9.92	0.0001
Error	94	15,400		
Total	96	18,650		
Parameters				
AUPC	1	1,187	7.25	0.008
Age	1	2,004	12.2	0.0007

FIGURE 3. Hemoglobin (Hb) (g/L) at study-end as a function of the area under parasitemia curve (AUPC) during five months. Adjusting for age, the attached table describes the regression procedure of Hb at end on AUPC and age. $AUPC = \Sigma [(D_{t+1} - D_t)(P_{t+1} + P_t)/2]$, where D = day and P = log parasite density.

sitemias were relatively more important than symptomatic parasite densities in determining the final Hb concentration.

Asymptomatic parasitemia may be beneficial in inducing and sustaining partial immunity against malaria.[30] However, a significant drawback is the suppression of Hb concentration. The absolute and prolonged impact of asymptomatic parasitemia on Hb levels could not be determined since no child was aparasitemic over the full study period. Moreover, all cohort children < 24 months were anemic. Hence, it was not possible to determine an asymptomatic parasitemia cut-off level which would hypothetically allow for semi-immunity to develop without leading to anemia.[22]

Hemoglobin levels only recovered when at least two of the measured malaria indices were ameliorated, i.e., when most of the malaria was eliminated (Table 4). This emphasizes the need for drugs to eliminate parasitemia for a certain period of time in order to improve anemia.[31] From this perspective, antimalarial treatment with drugs of short duration, e.g., chlorproguanil-dapsone or artemisinine derivatives, may be less effective against anemia under perennial transmission conditions because of more frequent symptomatic or asymptomatic re-infections than after treatment with drugs that are slowly eliminated. Even if such re-infections are adequately treated when symptomatic, there may be difficulties in restoring long-term Hb concentration, particularly in children < 24 months. Similarly, recurrent antimalarial drug treatment in small children with parasitemia, but no fever, may be successful in preventing anemia if performed with an antimalarial with relatively long action time, e.g., SP.[14,32] However, the effects of such repeated abortion of chronic asymptomatic parasitemia on the development of partial antimalarial immunity need to be considered.[33,34]

At study-end, the prevalence of parasitemia in children < 24 months old was lower than in those ≥ 24 months (Table 1). This possibly reflected the frequent use of SP treatment in small children, although Hb was lower in children < 24 months at study-end. This may be related to the fact that young children are more prone to develop anemia as a complication to malaria than their older peers. However, in the regression model of Figure 3, the two-way interaction term between age and parasitemia was not significant (data not shown), suggesting that at similar levels of parasitemia, the effect on Hb did not differ between ages. However, young age *per se* was also related to lower Hb, and since parasite densities are highest in youngest children, anemia in this age group may be an independent but additive result of low age and high parasite densities. In addition to malaria, other age-

related factors, e.g., iron deficiency or other infections, may also contribute to anemia. Hookworm infection, however, was rare and not related to Hb levels in this study, consistent with the age-dependent acquirement of infection.[35] Light hookworm infections have been implicated as a cause of anemia in populations with poor iron status, but in children < 3 years, hookworm egg counts rarely reach the concentration known to affect Hb concentration.[3,5] Although additional stool samples may have detected a few more infected children, in this cohort of anemic children hookworm infection was most likely of inferior importance.

In conclusion, our data from an area of high malaria transmission show that anemia in early childhood is highly associated to falciparum malaria but that the Hb value is influenced by several partly independent factors, i.e., a cumulative effect by continuous asymptomatic parasitemia, and an acute effect by a clinical episode, especially in connection with treatment failure. Prompt malaria case management, including sulfadoxine-pyrimethamine as a second-line drug, appears to restore short-term Hb values but does not, on its own, sufficiently improve the long-term Hb levels in children below 24 months of age.

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