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Age, temperature, and parasitaemia predict chloroquine treatment failure and anaemia in children with uncomplicated *Plasmodium falciparum* malaria

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Abstract

The prevalence of chloroquine-resistant *Plasmodium falciparum* malaria has been increasing in sub-Saharan Africa and parts of South America over the last 2 decades, and has been associated with increased anaemia-associated morbidity and higher mortality rates. Prospectively collected clinical and parasitological data from a multicentre study of 788 children aged 6–59 months with uncomplicated *P. falciparum* malaria were analysed in order to identify risk factors for chloroquine treatment failure and to assess its impact on anaemia after therapy. The proportion of chloroquine treatment failures (combined early and late treatment failures) was higher in the central-eastern African countries (Tanzania, 53%; Uganda, 80%; Zambia, 57%) and Ecuador (54%) than in Ghana (36%). Using logistic regression, predictors of early treatment failure included younger age, higher baseline temperature, and greater levels of parasitaemia. We conclude that younger age, higher initial temperature, and higher baseline parasitaemia predict early treatment failure and a higher probability of worsening anaemia between admission and days 7 or 14 post-treatment.

Keywords: malaria, *Plasmodium falciparum*, anaemia, chloroquine, treatment failure, Africa, Ecuador

Introduction

Plasmodium falciparum malaria is a major cause of morbidity and mortality worldwide, especially in sub-Saharan Africa. Levels of chloroquine resistance have steadily increased over the last 2 decades in Africa (Khan & Macguire, 1978; Brandling-Bennett *et al.*, 1988; Watkins *et al.*, 1988; Premji *et al.*, 1994; Bloland *et al.*, 1998; Makono & Sibanda, 1999) and in some areas of South America (Blair-Trujillo *et al.*, 2002; Marquiño *et al.*, 2003). The continued use of chloroquine as first-line therapy for malaria increases the risk of anaemia and the need for blood transfusions in young children (Greenberg *et al.*, 1989; Ekvall *et al.*, 1998; McElroy *et al.*, 2000). In Africa, chloroquine resistance has been associated with increased mortality, particularly in children aged < 5 years (Zucker *et al.*, 1996; Trape *et al.*, 1998).

Effective treatment of malaria reduces the risk of death in children with severe anaemia (Zucker *et al.*, 1996). In a community-based study of Tanzanian children, early treatment failure (ETF) with chloroquine was associated with a greater decrease in haemoglobin (Hb) level after 72 h than late treatment failure (LTF) or adequate clinical response (ACR). Despite ACR to chloroquine, no improvement in Hb levels was seen during follow-up greater than 21 d after initial therapy. In contrast, treatment with sulfadoxine–pyrimethamine (SP) resulted in significant improvement in Hb levels (Ekvall *et al.*, 1998).

Despite rising levels of resistance, chloroquine continues to be the antimalarial treatment of choice in many countries, because it is well tolerated, safe, inexpensive, and highly effective against susceptible strains of *P. falciparum*. Treatment with chloroquine often leads to rapid symptomatic improvement in patients with malaria despite failure of the drug to clear parasites from the blood (Hoffman *et al.*, 1984; Khoromana *et al.*, 1986; Brandling-Bennett *et al.*, 1988). Given the lack of inexpensive, non-toxic, and effective alternatives, the continued use of chloroquine in countries or regions with lower levels of resistance seems to be a reasonable approach until a better alternative becomes available and affordable.

In a study of children with uncomplicated *P. falciparum* malaria, conducted in the Solomon Islands, independent risk factors for chloroquine resistance included young age, high parasite density, normal spleen size, malnutrition, and the presence of gametocytes in thick films (Hess *et al.*, 1996). Using multivariate analysis, a recent study in Uganda found that age < 5 years, presenting temperature > 38.0 °C, and chloroquine use 3–4 d before study entry were independent predictors of chloroquine treatment failure (Dorsey *et al.*, 2000). While other studies have attempted to define risk factors for chloroquine treatment failure, methodological differences, small sample size, and failure to control for potentially confounding effects serve to limit the strength of their results (Brandling-Bennett *et al.*, 1988; Henry *et al.*, 1994, 1996; Alene & Bennett, 1996; Ekvall *et al.*, 1998). In addition, most studies have focused on only 1 country.

During the course of a randomized, placebo-controlled, multicentre trial of zinc as an adjunct to the treatment of acute, uncomplicated *P. falciparum* malaria in young children, data were prospectively collected on ETF and LTF with chloroquine (ZAP, 2002). This paper characterizes and contrasts chloroquine resistance *in vivo* in the 5 participating study sites and attempts to identify independent risk factors for chloroquine treatment failure and persistent anaemia after an episode of malaria.

Methods

Full details concerning the study design and methods can be found elsewhere (ZAP, 2002). In order to ensure uniformity of data collection across study sites, we used a common protocol, a detailed study

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procedure manual, pilot studies at each site, and technical site visits at the beginning of enrolment in order to assess and ensure uniformity of study procedures. Special quality control measures were used to ensure consistency in the performance and interpretation of blood films among the study sites. Internal quality control consisted of duplicate reading of a 10% subsample of smears by an experienced parasitologist at each site. while external quality control was done on a 5% sample of slides from all sites by an experienced parasitologist (Z. G. P.).

Study sites

Subject enrolment took place between December 1998 and May 2000 at the following locations: Hospital Delfina Torres (Esmeraldas, Ecuador), Komfo Anokye Teaching Hospital (Kumasi, Ghana), Kisarawe District Hospital (Kisarawe, Tanzania), Mpigi Health Center (Mpigi, Uganda), and Arthur Davison Children Hospital (Ndola, Zambia). These sites represent a mixture of urban and rural populations in Africa and Latin America. The 4 African sites are in malaria hyperholoendemic zones whereas the Ecuadorian site is in a hypoendemic zone.

Study subjects

Children aged 6–59 months with axillary temperature $\geqslant 37.5\,^{\circ}C$ and $\geqslant 2000/mm^3$ asexual forms of *P. falciparum* in a thick blood film were eligible for participation in the study. Exclusion criteria included: Hb < 70 g/L, severe malaria as defined by WHO (1990), non-*P. falciparum* or mixed *Plasmodium* infections, concurrent severe infections (i.e. lower respiratory infection, acute otitis media, pyelonephritis, typhoid fever, bloody diarrhoea, meningitis, or measles), severe dehydration, malnutrition as defined by the Wellcome criteria (Anonymous, 1970), inability to tolerate oral medications or fluids, chronic illness, and prior participation in the trial. Written informed consent was obtained from the child's parent or carer. The institutional review boards at each site and the Harvard School of Public Health, Boston, MA, USA, approved the study.

Once enrolled, children were admitted to an inpatient ward for 48–72 h. Demographic data, use of antivector measures to prevent malaria, recent antimalarial use, and significant past medical history were collected. A complete physical examination was performed at the time of study entry and daily during hospitalization and as clinically indicated. Axillary temperature was measured every 4 h. Paracetamol was administered for temperatures $\geqslant 38.5\,^{\circ}C$ every 6 h at a dose of 10–15 mg/kg.

Antimalarial treatment and follow-up

Chloroquine was the recommended first-line drug for the treatment of malaria in all 5 countries during the study period. The following dosing schedule was used: 10 mg/kg on day 0, 10 mg/kg on day 1, and 5 mg/kg on day 2. If either a treatment or parasitological failure occurred (see outcome classifications for definitions), treatment with a standard dose of either amodiaquine or SP (Fansidar, Roche, Zurich, Switzerland) was administered. Quinine was used for the treatment of children who developed severe malaria (WHO, 1990). Children with symptomatic severe anaemia were transfused with packed red blood cells. Discharge from hospital occurred once the fever had resolved (axillary temperature < 37.5 °C for at least 12 h) and there was a $\geqslant 75\%$ reduction of parasitaemia relative to baseline.

Children were seen at follow-up at the health centre on days 7, 14, and 28. If the patient did not return for follow-up, a research assistant went to the subject's house to locate the patient. At each follow-up visit, a history of fever during the last week and recent antimalarial drug use were collected, blood smears were performed, and Hb was measured.

Laboratory tests

Giemsa-stained thick blood films were prepared at the time of enrolment, at 24 h, 36 h, 48 h, 72 h, and at days 7, 14, and 28. Total parasite count per microlitre was quantitated by counting the number of parasites per 200 white blood cells (WBC), assuming a total WBC count of 8000/μL (Trape, 1985). A thick film was declared negative after viewing high power fields containing 500 WBCs. The Hb concentration was determined by HemoCue (Angelholm, Sweden) on admission and on days 7, 14, and 28.

Outcome classification

Therapeutic response was defined using a modified version of the WHO 14-day *in vivo* clinical classification system (WHO, 1996; Table 1). The definition of LTF was modified as follows. Because axillary temperatures were not taken during follow-up home visits, we used a history of fever during the last week instead of axillary temperature and history of fever in the previous 48 h. We also used a history of hospitalization with parasitaemia in place of severe malaria/danger signs with parasitaemia. The response to chloroquine treatment was also classified according to the WHO parasitological classification system (WHO, 1973; Table 1).

Data management and statistical methods

Data entry and management was carried out using Epi-Info, version 6.04c (CDC, Atlanta, GA, USA) and Integrated Microcomputer Processing System, version 3.1 (US Bureau of Census, Washington, D.C., USA). Since there was no effect of the zinc supplement on clinical, parasitological, or haematological outcomes (ZAP, 2002), the 2 treatment groups were combined for the purposes of this analysis.

Statistical analyses were carried out using SAS, version 8.2 (SAS Institute Inc., Cary, NC, USA). Odds ratios and 95% CIs were predicted from logistic regression parameter estimates and Wald confidence intervals. Multiple logistic regression models for predictors of ACR, ETF, and LTF created models that initially included study site, age in months, use of bednets, initial temperature, log-transformed baseline parasitaemia, baseline Hb, gender, use of personal antimosquito control, use of antimalarials in the previous 7 d, breastfeeding, mother literate, weight-for-age *Z*-score, height-for-age *Z*-score, and whether the subject was randomized to receive zinc.

Predicators of Hb change were modelled with multivariate, repeated measures analysis of variance (ANOVA). The model initially included an indicator variable for ACR, study site, age in months, use of bednets, initial temperature, log-transformed baseline parasitaemia, gender, use of personal antimosquito control, use of antimalarials in the previous 7 d, breastfeeding, mother literate, weight-for-age *Z*-score, height-for-age *Z*-score, and whether the subject was randomized to receive zinc.

Both final models were constructed using a backwards elimination selection process and these models were confirmed with a forward selection process. All models included indicator variables for study site. Two-way interactions of statistically significant main effects were tested.

Results

Baseline characteristics

One thousand and eighty-seven children were enrolled. The analytical data set consisted of the 788 children for whom complete clinical and parasitological data were available. Baseline characteristics of the study participants, broken down by site of enrolment, are

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Table 1. Definitions of clinical and parasitological outcomes in study children with uncomplicated *Plasmodium falciparum* malaria

	Definition
Clinical outcome	
Early treatment failure (ETF)	Any of the following occurring on days 1–3: 1. Development of severe malaria/danger signs (unconsciousness, prostration, convulsions, persistent vomiting, severe anaemia) with parasitaemia 2. Axillary temperature ≥ 37.5 °C on day 2 with parasite density > day 0 parasite density 3. Axillary temperature ≥ 37.5 °C on day 3 with parasitaemia 4. Day 3 parasite density > 25% day 0 parasite density
Late treatment failure (LTF)	Any of the following occurring on days 4–14: 1. Hospitalization with parasitaemia 2. Report of fever since last visit with parasitaemia
Adequate clinical response (ACR)	1. Absence of parasitaemia on day 14 irrespective of axillary temperature without meeting any of the criteria of ETF or LTF 2. No report of fever irrespective of the presence of parasitaemia, without meeting any of the criteria of ETF or LTF
Parasitological outcome	
Sensitivity (S)	Asexual parasitaemia is reduced to 25% or less of the original pre-test level during the first 48 h of treatment. Asexual parasitaemia clears by day 7 and does not reappear
RI	Asexual parasitaemia is reduced to 25% or less of the original pre-test level during the first 48 h of treatment. Asexual parasitaemia clears by day 7, but reappears on either day 14 or day 28
RII	Asexual parasitaemia is reduced to 25% or less of the original pre-test level during the first 48 h of treatment. Asexual parasitaemia never clears
RIII	Asexual parasitaemia is reduced by less than 75% during the first 48 h or it continues to rise

displayed in Table 2. We compared the baseline demographic, clinical, and parasitological characteristics of the group of children who were excluded from analysis with the group for whom we had complete data, and found few differences. There was greater bednet use in the group with complete data. Subjects in Ecuador tended to be older (mean age 39.2 months) than those in the African sites (mean age 25.4 months) and had significantly lower geometric mean parasite counts (9450 vs. 19 930/μL). Lastly, children in Uganda had a lower mean height-for-age *Z*-score.

The children enrolled from Tanzania and Zambia had higher levels of parasitaemia than those from Ghana, Uganda, or Ecuador. Baseline Hb levels and bednet use varied widely across sites. The reported use of antimalarials in the week before hospitalization varied from 17% in Ecuador to 54% in Uganda. Chloroquine was by far the most commonly used antimalarial overall (92% overall).

Clinical and parasitological outcomes

Chloroquine treatment failures were common in Central-East Africa and Ecuador with ETFs ranging from 38 to 65%. Significantly more children in Ghana had ACRs than children in the other 4 countries combined ($P < 0.0001$). Parasitological resistance results are displayed in Table 3. Combined RII and RIII resistance was > 50% in all countries except Ghana (48.7%). A moderate number of discordant results were encountered when parasitological resistance rates were compared with ETF and LTF (Table 4). We combined S and RI (which are closest to the definition of ACR) and computed a κ statistic to compare the concordance between the 2 classification systems. The agreement between the 2 systems was 67% and had a κ coefficient of 0.48 ($P < 0.0001$).

ETF occurred in 17.1% (48/280) of children with RI or S parasitological outcomes. Although there appeared to be discordance between the presence of RIII parasitological resistance and an ACR in 52 children, this discrepancy proved to be a result of how RIII was defined. All 52 children failed to have a reduction to 25% of baseline parasitaemia by 48 h (therefore meeting the definition of RIII) but, by 72 h, parasitaemia had dropped below 25% (thus not fulfilling the criteria for ETF). Surprisingly, 20.2% (68/337) of children with ACRs had RII resistance.

Predictors of treatment failure

Logistic regression analysis was used to identify possible predictors of chloroquine treatment failure (combined ETF and LTF). Univariate predictors of ETF included younger age, increased temperature at baseline, higher parasitaemia at presentation, and current breastfeeding. Gender, zinc treatment, bednet use, use of antivector measures, height-for-age *Z*-score, weight-for-age *Z*-score, baseline zinc plasma levels, and history of recent antimalarial therapy were not significantly associated with treatment failure. The risk of chloroquine treatment failure increased with decreasing age, higher baseline temperature, and greater density of baseline parasitaemia (Table 5). In the presence of the variable age, breastfeeding was not a predictor of treatment failure. A full model was fitted, controlling for study site, gender, zinc treatment, bednet use, use of antivector measures, height-for-age *Z*-score, weight-for-age *Z*-score, baseline zinc plasma levels, and history of recent antimalarial therapy. However, the inclusion of the additional terms had only minor impact on the odds ratios and 95% CIs.

Haematological outcomes

Most subjects (84%) were anaemic (Hb < 110 g/L) at baseline. In the overall study cohort, Hb levels declined between days 0 and day 7 from a mean of 92.6 g/L to 87.2 g/L. Subsequently, the Hb concentration began to rise, reaching a mean of 95.5 g/L at day 14 and 100.9 g/L at day 28. The final value at day 28 was a mean of 8.3 g/L, higher than at the time of presentation to the hospital. Children who had an ACR

Table 2. Baseline characteristics of study children with uncomplicated *Plasmodium falciparum* malaria

	Ecuador	Ghana	Tanzania	Uganda	Zambia
No. enrolled	71	131	182	180	224
Mean ± SEM age (months)	39.2 ± 1.68	27.2 ± 1.28	26.0 ± 1.25	22.0 ± 1.1	26.7 ± 1.04
Gender (% male)	47.9	50.4	49.5	51.1	53.1
Breastfeeding (%)	5.6	34.4	48.9	45.0	42.4
Mean ± SEM years of carer's education	6.3 ± 0.47	6.2 ± 0.42	5.6 ± 0.25	5.2 ± 0.23	5.5 ± 0.23
Bednet use by child (%)	91.5	10.7	54.9	1.7	6.3
Antimalarial use in the past 7 d (%)	16.9	21.4	42.3	54.4	22.8
Mean ± SEM weight-for-age *Z*-score	−0.8 ± 0.12	−1.2 ± 0.08	−1.1 ± 0.08	−1.1 ± 0.10	−1.3 ± 0.07
Mean ± SEM height-for-age *Z*-score	−0.7 ± 0.14	−1.3 ± 0.11	−1.1 ± 0.12	−2.2 ± 0.12	−1.4 ± 0.08
Mean ± SEM axillary temperature (°C)	38.3 ± 0.12	38.6 ± 0.08	38.5 ± 0.06	38.4 ± 0.06	39.0 ± 0.06
Splenomegaly (%)	28.2	13.0	7.7	19.4	31.7
Mean ± SEM haemoglobin (g/L)	102.0 ± 1.73	93.5 ± 1.28	87.4 ± 1.06	91.7 ± 1.28	93.2 ± 1.10
Anaemia (Hb < 110 g/L) (%)	92.3	81.1	85.3	82.4	69.0
Geometric mean parasitaemia (range)	9450 (2000–126640)	13891 (2000–238400)	35875 (2000–435920)	7539 (2000–62080)	37073 (1500–415200)

to chloroquine had significantly higher Hb concentrations at days 7 ($P < 0.0001$) and 14 ($P = 0.0057$) when compared with those with ETF or those with combined ETF and LTF (Figure). The day 28 Hb level was higher in children who had an ACR but the difference was not statistically significant ($P = 0.84$).

Predictors of differences in haemoglobin from day 0 to 28

Risk factors for differences in Hb between the initiation of therapy and day 28 were identified using a repeated measures ANOVA. Predictors of differences in Hb included age ($P < 0.0001$), initial parasitaemia ($P = 0.0004$), and recent antimalarial therapy ($P = 0.0073$) controlling for initial temperature, study site, gender, zinc treatment, bednet use, other antivector measure use, and ACR.

Discussion

Our study demonstrated high rates of chloroquine treatment failure in Ecuador, Tanzania, Uganda, and Zambia and moderate levels of resistance in Ghana. Only 20% of children in Uganda and less than half the children in Ecuador, Tanzania, and Zambia had an ACR to chloroquine. Although Ghanaian children had better clinical responses (64.1%), the treatment failure rate was nonetheless above the 25% threshold recommended by the WHO for a change in national drug policy (WHO 1996).

The rate of resistance encountered in Uganda was higher than recently described. Dorsey *et al.* (2000) found a treatment failure rate of 47% compared with 80% in our study. One possible explanation is that the Dorsey study included adults who are more likely to have underlying antimalarial immunity. The ability to clear chloroquine-resistant parasites after treatment improves with age (Djimbé *et al.*, 2001).

The chloroquine resistance rates in Tanzania were similar to those that have been previously reported (Premji *et al.*, 1994; Ekvall *et al.*, 2001). Similarly, high rates of chloroquine treatment failures have also been described in Zambia (Barat *et al.*, 1998). At the time that we carried out this study, the Ministries of Health of all of the 5 participating countries were still recommending the use of chloroquine as a first-line agent for the treatment of uncomplicated malaria in children. The results of our study provide further data to uphold the decisions that have subsequently been made by the Ministries of Health in Tanzania, Uganda, and Zambia to change from chloroquine as first-line therapy (MoH, Tanzania, 1999; MoH, Uganda, 2001; MoH, Zambia, 2002). In Tanzania, although the Ministry of Health officially recommended discontinuing the use of chloroquine as the first-line drug, there continues to be extensive use of chloroquine in non-governmental health facilities and private practice associated with practitioner and community concerns about SP safety and cost. Similarly, in Zambia, although an official recommendation to change malaria drug policy has been made, the process of conversion to SP as an intermediate strategy prior to the introduction of artesunate-based combination therapy is proving to be logistically complicated. Thus, the findings of our study remain relevant to these countries.

The κ coefficient of 48% represents a moderate agreement above chance between the 2 classification systems, indicating that we found a moderate level of discordance between the presence of parasitological resistance and clinical outcomes, especially when resistance was compared with ACRs. Some of this discrepancy might have stemmed from our relatively strict adherence to standard WHO definitions (WHO, 1973, 1996). Many children had evidence of RI or RII resistance but nevertheless had ACRs. Similar findings were noted in a study of chloroquine resistance in Mali in which children aged < 5 years were more likely than older persons to have discordance between ACR and

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Table 3. Parasitological outcomes in study children with uncomplicated *Plasmodium falciparum* malaria

Study site	No.	RIII (%)	RII (%)	RI (%)	S (%)
Ecuador	71	38.0	36.6	19.7	5.6
Ghana	131	22.1	26.7	20.6	30.5
Tanzania	182	36.3	20.3	23.6	19.8
Uganda	180	62.8	28.3	7.2	1.7
Zambia	224	39.3	16.1	24.1	20.5
All Sites	788	40.9	23.5	19.2	16.4

Table 4. Comparison of clinical and parasitological outcomes in study children with uncomplicated *Plasmodium falciparum* malaria for all study sites

Outcome	RIII	RII	RI	S	Total
Early treatment failure	254	59	30	18	361
Late treatment failure	17	58	15	0	90
Adequate clinical response	52	68	106	111	337
Total	323	185	151	129	788

Table 5. Multivariate regression analysis of risk of independent predictors of chloroquine treatment failure in study children with uncomplicated *Plasmodium falciparum* malaria controlling for study site

Variable	Odds ratio	95% CI	*P*
Age per month increase	0.97	0.96–0.98	<0.0001
Temperature per 1 °C increase	1.28	1.062–1.545	<0.0098
Parasitaemia per 10-fold increase	1.21	1.07–1.36	0.0019

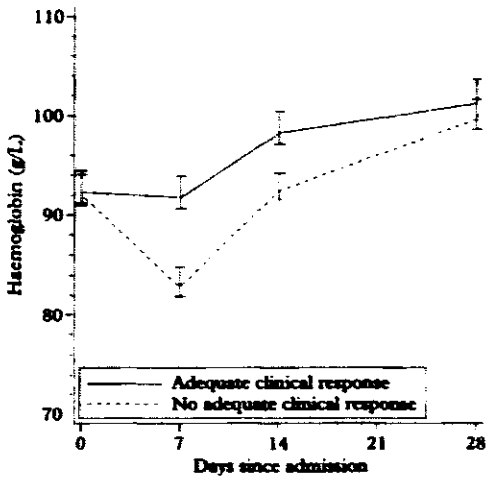

Figure. Mean haemoglobin concentration in study children with uncomplicated *Plasmodium falciparum* malaria with and without adequate clinical response between days 0 and 28. Error bars indicate 95% CI.

RI or RII resistance (Plowe *et al.*, 2001). A recent study in Ghana also demonstrated this discordance (Ehrhardt *et al.*, 2002). We encountered high rates of ETFs, which were usually associated with RII or RIII resistance. Only a small proportion of children had S or RI parasitological outcomes in the setting of early treatment failures. We also found that a small number of children with an ACR had RII or RIII resistance. Although our results are substantially different from those of Plowe *et al.* (2001), this may have been due to the higher levels of parasitological resistance and treatment failures present at our study sites. In Plowe's report, younger children were more likely to have discordance between ACR and evidence of parasitological resistance (Plowe *et al.*, 2001). Since our study population only included children aged < 5 years, the greater degree of discordance between the parasitological resistance and therapeutic *in vivo* efficacy outcomes may have been largely due to the age range of our study population.

Younger age, higher temperature, and greater density of parasitaemia were independent predictors of chloroquine treatment failure in our study. Young age and high parasite density have been demonstrated in previous studies to be predictors of antimalarial drug resistance (Hess *et al.*, 1996; Dorsey *et al.*, 2000; Ehrhardt *et al.*, 2002). A presenting temperature > 38 °C was a predictor of treatment failure in Uganda (Dorsey *et al.*, 2000). Our study also demonstrated that higher temperature was associated with an increased risk of chloroquine treatment failure. In addition, we demonstrated a progressive increase in the likelihood of treatment failure with rising temperature. A physiological explanation for the association between higher baseline temperature and risk of treatment failure is not apparent.

Recent antimalarial treatment, which was predominantly chloroquine, was not associated with an increased risk of treatment failure in our study. In contrast, Dorsey *et al.* (2000) found that patients who had taken chloroquine 3–14 d prior to study entry were more likely to be treatment failures when compared with patients who had not recently used chloroquine or who had been treated with chloroquine in the last 3 d (Dorsey *et al.*, 2000).

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Two limitations of our study were the lack of urine testing for chloroquine and its metabolites and failure to define precisely when chloroquine was taken prior to presentation at the health centre. The majority of the carers of the children in our study denied recent antimalarial use. Previous studies have shown a high correlation between admitting to have taken an antimalarial and a positive urine test (Dorsey *et al.*, 2000). However, the reliability of a patient's denial of recent antimalarial use is lower as many of these patients have detectable drug or metabolites in their urine (Dorsey *et al.*, 2000). The lack of reliability of a negative report of recent antimalarial use complicates the interpretation of our data, which relied on carer recall. In addition, we did not ask the carers of the children in our study to define when antimalarials were taken. As a consequence, we cannot conclusively state that recent chloroquine use is not a risk factor for treatment failure. Since recent chloroquine use has been associated with parasitological resistance in other studies (Henry *et al.*, 1996; Bayoumi *et al.*, 1997), there appears to be a reasonable probability that this is also a predictor of treatment failure.

Another potential criticism of our study was our decision to not take temperatures at days 7 and 14 in accordance with WHO guidelines (WHO, 1996). We relied instead on a report of recent fever. A negative report of fever has been shown to have a high negative predictive value whereas a positive response is of questionable reliability (Einterz & Bates, 1997, 1998; Verhoef *et al.*, 1998). Not measuring temperature on days 7 and 14 should only have had an impact on the outcome, LTF. However, the independent risk factors for treatment failure in our cohort of children (young age, higher temperature, and higher parasite density) were most strongly associated with ETF. As a consequence, the use of recall data for recent fever is unlikely to have had a meaningful impact on our study findings.

Our entire study cohort was found to have worsening anaemia between baseline and day 7 with a gradual recovery of Hb over the following 3 weeks. Predictors of slower recovery of Hb in our study included younger age, higher presenting temperature, and greater parasite density. In children with ETF, there was a delayed recovery of Hb relative to those children who had ACRs. A recent study in Tanzania also found that children with ETF had a significantly greater decrease in Hb level at 72 h (Ekvall *et al.*, 1998). However, in contrast to our study, which demonstrated a rise in Hb level by days 14 and 28 to levels that were higher than at the time of hospital presentation, the Tanzanian study found that all children who were treated with chloroquine did not recover to pre-treatment Hb levels. Differences in the dietary intake of iron, phytates, and the prevalence of concomitant parasitic infections (e.g. hookworm infections) are potential explanations for the difference between our results and those of the Tanzanian study.

The strengths of our study included a large sample size and the use of a consistent protocol with multiple quality control measures simultaneously in several different countries with varying degrees of transmission, with resultant variable underlying partial immunity of the population. The independent predictors of chloroquine treatment failure derived from our results and those of others (Hess *et al.*, 1996; Ekvall *et al.*, 1998; Dorsey *et al.*, 2000) can be used to risk-stratify children who might benefit from immediate use of second-line antimalarials in countries with high levels of chloroquine resistance. Because of the clear association between treatment failure and delayed recovery from anaemia, the association between repeated episodes of malaria and anaemia, known negative impact of anaemia on physical activity and cognitive development in young children, and the need to reduce anaemia-associated blood transfusions in areas where hepatitis and HIV are risks, countries that continue to use chloroquine in the face of treatment failure rates > 25% must carefully re-evaluate their first-line treatment policies.

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Announcement

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