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FACTORS CONTRIBUTING TO ANEMIA AFTER UNCOMPLICATED FALCIPARUM MALARIA

RIC N. PRICE, JULIE A. SIMPSON, FRANÇOIS NOSTEN, CHRISTINE LUXEMBURGER, LILI HKIRJAROEN, FEIKO TER KUILE, TAN CHONGSUPHAJASIDDHI, and NICHOLAS J. WHITE

Shoklo Malaria Research Unit, Mae Sod, Tak Province, Thailand; Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; Department of Infectious Diseases and AIDS, Academic Medical Centre, University of Amsterdam, The Netherlands; Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Headington, Oxford, United Kingdom

Abstract

The factors contributing to anemia in falciparum malaria were characterized in 4,007 prospectively studied patients on the western border of Thailand. Of these, 727 patients (18%) presented with anemia (haematocrit < 30%), and 1% (55 of 5,253) required blood transfusion. The following were found to be independent risk factors for anemia at admission: age < 5 years, a palpable spleen, a palpable liver, recrudescence of infections, being female, a prolonged history of illness (> 2 days) before admission, and pure *Plasmodium falciparum* infections rather than mixed *P. falciparum* and *Plasmodium vivax* infections. The mean maximum fractional fall in hematocrit after antimalarial treatment was 14.1% of the baseline value (95% confidence interval [CI], 13.6–14.6). This reduction was significantly greater in young children (aged < 5 years) and in patients with a prolonged illness, high parasitemia, or delayed parasite clearance. Loss of parasitized erythrocytes accounted for < 10% of overall red blood cell loss. Hematological recovery was usually complete within 6 weeks, but it was slower in patients who were anemic at admission (adjusted hazards ratio [AHR], 1.9, 95% CI, 1.5–2.3), and those whose infections recrudesced (AHR, 1.2, 95% CI, 1.01–1.5). Half the patients with treatment failure were anemic at 6 weeks compared with 19% of successfully treated patients (relative risk, 2.8, 95% CI, 2.0–3.8). Patients coinfecting with *P. vivax* (16% of the total) were 1.8 (95% CI, 1.2–2.6) times less likely to become anemic and recovered 1.3 (95% CI, 1.0–1.5) times faster than those with *P. falciparum* only. Anemia is related to drug resistance and treatment failure in uncomplicated malaria. Children aged < 5 years of age were more likely than older children or adults to become anemic. Coinfection with *P. vivax* attenuates the anemia of falciparum malaria, presumably by modifying the severity of the infection.

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Reprint requests: R. N. Price, Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Headington, Oxford, United Kingdom, Telephone: +44-1865-220970, Fax: +44-1865-2220984 (ricprice@doctors.org.uk).
Authors' addresses: Ric N. Price, Feiko ter Kuile, Christine Luxemburger, Lili Hkirjaroen, and François Nosten, Shoklo Malaria Research Unit, P.O. Box 46, Mae Sod 63110, Tak Province, Thailand. François Nosten, T. Chongsuphajaisiddhi, Julie A. Simpson, and Nicholas J. White, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. Feiko ter Kuile, Department of Infectious Diseases and AIDS, Academic Medical Centre, University of Amsterdam, The Netherlands. Ric N. Price, C. Luxemburger, François Nosten, and Nicholas J. White, Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Headington, Oxford, United Kingdom.

INTRODUCTION

Malaria is a significant cause of anemia in the tropics, particularly in children.¹ The relationship between the severity and duration of anemia and the intensity of infection is complex. Anemia results from the destruction of parasitized erythrocytes, the shortened survival of unparasitized erythrocytes, and a variable degree of bone marrow dyserythropoiesis.² In general, the more severe the infection, the more profound the anemia.³ The importance of drug-resistant malaria as a cause of anemia has been highlighted in recent years as chloroquine resistance has increased in Africa⁴; the incidence of severe anemia requiring hospitalization and the need for blood transfusions have increased in parallel.⁵⁻⁷ In high-transmission areas, where people are infected repeatedly, the contribution of an individual infection to anemia may be difficult to determine. The interaction between malaria and anemia has been less well studied in areas of low or unstable transmission. We report here on the relationship between anemia and falciparum malaria on the western border of Thailand, where transmission is low and multidrug resistance in *Plasmodium falciparum* deteriorated steadily during the past 10 years.

PATIENTS AND METHODS

Study site

This review is based on a series of large prospective chemotherapeutic trials aimed at optimizing antimalarial treatment regimens in an area of multidrug-resistant falciparum malaria. These studies took place between 1990 and 1995 in a Karen community, the members of which were living in 2 camps for displaced people located in an area of malarious hill forest on the western border of Thailand. Studies involving 4,551 of the patients described here have been reported previously.⁹⁻¹⁶ Transmission of malaria in this area is low (each person experienced approximately one bout of vivax and one bout of falciparum malaria infection every 2 years) and seasonal.⁸ Nearly all falciparum malaria infections become symptomatic, and hence the treatment of every episode could be documented reliably.

Between May 1990 and April 1992, a prospective malaria epidemiological study was conducted in Shoklo (one of the camps) with a population at midstudy estimated at 4,728; in total, 5,776 cases of falciparum malaria were diagnosed, and 400 (7%) of these patients were hospitalized. Thus, the overall (95% confidence interval [CI]) incidence of symptomatic falciparum malaria was 611 (601–621) per 1,000 people per year.⁸ This estimate incorporates both primary and recrudescence attacks. Of the 400 patients who were hospitalized, 303 patients (76%) were considered to have severe malaria, and 11 (3%) died. Thus, malaria is an important cause of morbidity and mortality in the area, and it is a major contributor to anemia, although hookworm, iron deficiency, and thalassemia also occur.

Patients

All patients with uncomplicated falciparum malaria who were enrolled into therapeutic studies at the Shoklo Malaria Research Unit between 1990 and 1995 were included in the analysis. Patients of all ages were eligible to enroll in these prospective chemotherapeutic

studies if they presented with slide-confirmed falciparum malaria to the Shoklo Malaria Research Unit or the outpatient clinics of Médecins Sans Frontières.

Patients were recruited into prospective studies provided that they or their accompanying relatives gave fully informed consent. Pregnant women, children weighing < 5 kg, and patients with concomitant disease requiring hospital admission were all excluded.

Therapeutic studies in Shoklo were carried out in 3 prospectively defined patient groups because each of these required a different treatment approach: 1) primary episodes of falciparum malaria; 2) recrudescence cases of malaria (recurrence of malaria within 63 days of a previous episode); and 3) hyperparasitemic falciparum malaria (> 4% parasitized red blood cells [RBCs]). Apart from this categorization, the inclusion and exclusion criteria were identical between studies. All studies were approved by the ethics committee of the Faculty of Tropical Medicine, Mahidol University, and the Karen Refugee Committee.

Clinical and laboratory procedures and patient follow-up were similar irrespective of whether they were enrolled into a comparative drug study or monitored routinely. At admission, a full clinical examination was completed, and capillary blood was drawn for routine hematology. Hematocrit was measured from 100 μ L heparinized capillary blood, and packed cell volume was measured by microcentrifugation. Parasite counts were determined on Giemsa-stained thick and thin blood films. If the count on the thick film exceeded 1,000 parasites per 500 white blood cells, the thin film result (expressed as the number of erythrocytes per 1,000 RBCs) was recorded.

Treatment with one of the drug regimens (Table 1) was assigned to patients in comparative randomized studies as described previously.⁹⁻¹⁵ For the purposes of this analysis, they were then categorized into 4 treatment groups: mefloquine (single or split dose without artemisinin derivatives), halofantrine (high or low dose), artemisinin derivatives (artesunate or artemether with and without mefloquine), and quinine. Primaquine was not given routinely to eradicate the liver stages of *Plasmodium vivax* in mixed infections.

Drug administration was observed in all patients. All patients were examined daily until they became asymptomatic and a parasitemic; they were then seen weekly during the follow-up period; before 1993, this was for 4 weeks, and after 1993, it was for 9 weeks. A malaria blood smear and blood for hematocrit was taken at each weekly visit, or if symptoms returned, between follow-up appointments.

After recovery from the acute episode of malaria, patients with a hematocrit of < 30% were prescribed a 2-week course of unsupervised ferrous sulfate and folic acid supplementation. Patients presenting with symptomatic and severe anemia (defined as hematocrit < 20%) were transfused. A cross-sectional malariometric survey of randomized houses in this camp was carried out in 1994.⁸

Data analysis

Data were analyzed by the statistical programs SPSS for Windows (SPSS Inc., Chicago, IL) and Epi Info 6 (CDC, Atlanta, GA). Normally distributed continuous data were compared by Student's *t*-test and analysis of variance. Data not conforming to a normal distribution were

compared by the Mann-Whitney *U* test and Kruskal-Wallis analysis of variance. The association between 2 continuous variables was assessed by Spearman's rank correlation coefficient.

Anemia was defined prospectively as a hematocrit < 30%, and further classified as severe if the hematocrit was < 20%. In patients whose infections did not recrudescence during follow-up, the fractional reduction in hematocrit attributable to malaria was defined before and after treatment as the difference between the patient's "normal" hematocrit on Day 42 and that on Day 0 or Day 7, respectively (see Figure 1 and Results) divided by the hematocrit on Day 42.

Patients were stratified prospectively into the following age groups: young children (aged < 5 years), older children (aged 5–14 years), and adults (aged > 14 years). Proportions were compared by calculating by the chi-square test with Yates' correction or by Fisher's exact test. Survival analysis was used to assess risk factors associated with the development of anemia during the follow-up period (for those patients not anemic at admission) and the time taken to return to a normal hematocrit (defined for each age and sex group as a hematocrit above the 25th percentile of the population hematocrit on Day 42 after antimalarial treatment) (Table 2). Cumulative incidences were calculated by the product limit method and compared by the Mantel-Haenszel log rank test. When assessing independent risk factors, a Cox regression model was used.

A multiple logistic regression model and a multiple linear regression model were used to determine adjusted odds ratios for risk factors for anemia at admission and adjusted regression coefficients for the variables associated with the fall in hematocrit attributable to malaria, respectively.

In all multivariate analyses, independent risk factors were assessed after stratifying by the 4 treatment categories to account for potential drug effects. Any variable found to be associated significantly with anemia in a univariate analysis was entered into the equation and the model constructed by a forward stepwise analysis of the Wald statistic.

RESULTS

Between November 1990 and May 1995, 5,253 patients (2,328 female subjects and 2,925 male subjects) were enrolled into drug studies. Of these patients, 3,148 (60%) were recruited into randomized prospective studies and a further 2,105 (40%) patients presented to the clinics. Although the latter group was not part of comparative studies, they were treated in the same way and therefore included in this analysis. In total, 3,848 patients (73%) had primary infections and 1,405 (27%) had recrudescences of earlier falciparum malaria. There were 865 patients (16.5%) aged < 5 years, 2,425 (46%) aged 5–14 years, and 1,963 (37%) aged > 14 years old. The geometric mean parasitemia at admission was 5,347 parasites/ μ L (95% CI, 5,047–5,664) (Table 3). Follow-up was achieved in 4,294 (82%) patients to Day 28 and 2,599 (50%) to Day 42 after the start of antimalarial treatment.

Hematological responses

Hematocrit data were available for 4,007 patients at admission, and the subsequent analysis in this section is confined to these patients. Of these, 3,328 patients (84%) had pure *P. falciparum* malaria and 634 (16%) had mixed *P. falciparum* and *P. vivax* infection. Mixed infections occurred in 22.8% (194 of 852) of young children (< 5 years old) compared with 15.3% (659 of 4,316) in older patients ($P < 0.001$). The mean hematocrit at admission was 34.7% (95% CI, 34.5–34.9). Anemia (hematocrit < 30%) occurred in 18% (727 of 4,007) patients. The incidence of anemia in falciparum malaria was highest in infants (56%). Admission characteristics are summarized in Table 3. The hematocrit at admission was correlated positively with age in children ($r_s = 0.32$; $P < 0.001$) but not in adults, and overall showed a very weak positive correlation with parasitemia ($r_s = 0.05$; $P = 0.001$).

In a multivariate model, after correcting for baseline parasitemia, the following were found to be independent risk factors for anemia (defined as a hematocrit < 30%) at admission: 1) children aged < 5 years compared with children aged 5–14 years (adjusted odds ratio [AOR], 2.6, 95% CI, 1.6–6.5; $P < 0.001$); and 2) children aged < 5 years compared with patients > 14 years of age (AOR, 3.6, 95% CI, 1.8–81; $P < 0.001$), a palpable spleen (AOR, 2.3, 95% CI, 2.1–2.5; $P < 0.001$), a palpable liver (AOR, 2.0, 95% CI, 1.7–2.2; $P < 0.001$), recrudescence infections (AOR, 1.9, 95% CI, 1.7–2.1; $P < 0.001$), being female (AOR, 1.5, 95% CI, 1.3–1.5; $P < 0.001$), a prolonged history of illness before admission (> 2 days) (AOR, 1.5, 95% CI, 1.3–1.7; $P < 0.001$), and pure *P. falciparum* infections rather than mixed *P. falciparum* and *P. vivax* infections (AOR, 1.3, 95% CI, 1.1–1.6; $P = 0.04$).

Hematological recovery

Overall, 55 (1%) patients required a blood transfusion. These patients were excluded from the analysis when assessing hematological profiles after admission. Since July 1993, individual patient follow-up has lasted 42–63 days. In the 912 patients followed for longer than 42 days, no significant change in hematocrit was observed after Day 42, suggesting complete hematological recovery by this time (Figure 1). The Day 42 hematocrit was therefore taken as the normal value for that person. In total, only 1,372 (26%) patients had available hematocrit values on Day 42, and most of the data available came from those who received mefloquine plus 3 days of artesunate (MAS3) treatment (60%). A multivariate model was then generated with only MAS3-treated patients to predict the hematocrit value on Day 42 and thus that person's normal hematocrit, from the admission parasitemia and hematocrit values on Day 0, Day 7, and Day 28 (adjusted $R^2 = 0.33$). The model was tested in 283 patients who did not receive MAS3 but who had their hematocrit measured on Day 42; we found no significant difference in the actual and predicted Day 42 hematocrit value (median difference, 0.06%, interquartile range [IQR], –2.0 to 2.0%). Data on a further 779 patients were generated by use of the model. Pooling actual and predicted Day 42 hematocrit levels demonstrated no difference in their age-stratified mean values and those obtained from a cross-sectional hematocrit survey of the study population (Table 2). These observations supported the use of the Day 42 hematocrit as representing full hematological recovery from uncomplicated malaria.

Malaria attributable anemia

Before treatment—Before treatment, the median fractional reduction fall in hematocrit attributable to malaria was 2.3% (IQR, −5.7 to 10.6%). This reduction was correlated weakly and negatively with both the parasitemia at admission ($r = -0.06$, $P < 0.01$) and age ($r_s = -0.14$, $P < 0.001$). Children aged < 5 years had a median (IQR) fractional fall in hematocrit of 7.5% (−3.1 to 17.5%) compared with 1.4% (−6.2 to 9.2%) in older children and 1.4% (−6.3 to 9.2%) in adults ($P < 0.001$). A severe reduction in hematocrit (defined as a fractional fall $> 25\%$) occurred in 13% (45 of 354) of patients aged < 5 years, compared with 4% (44 of 1073) in older children and 3.2% (21 of 649) in adults ($P < 0.001$). In a multivariate model, age < 5 years, recrudescence infection, and a palpable spleen or liver were independent risk factors for a fractional reduction in hematocrit $> 25\%$, and together they explained 60% of the cases (Table 4).

After treatment—The median (IQR) fractional fall in hematocrit attributable to malaria after treatment started was 8.9% (2.7–15.4%). The fractional fall in hematocrit was correlated positively with the admission parasite count ($r_s = 0.25$, $P < 0.001$). Once again, the fall was greatest in the youngest age group (11.4; 5.5–19.4%) compared with 9.3 (3.2–15.6%) in older children and 6.6 (0.0–13.8%) in adults ($P = 0.001$). Overall, 12.4% (37 of 298) of patients aged < 5 years had a large fractional fall ($> 25\%$) compared with 5.4% (50 of 930) in older children and 5.2% (31 of 595) in adults ($P < 0.001$). In a multivariate model age, prolonged preadmission history of fever, high initial parasitemia, and delayed parasite clearance were all found to be significant independent risk factors. Together they explained 84% of cases in which the fractional fall in hematocrit exceeded 25% (Table 4).

The overall mean maximal fractional reduction in hematocrit (including both pre- and posttreatment falls) resulting from acute uncomplicated falciparum malaria in this series was 14.1% (95% CI, 13.6–14.6). The effect of age on the maximal fractional drop in hematocrit proved independent of all other confounding factors ($P = 0.004$) (Figure 2).

Contribution of the loss of parasitized RBCs to anemia—From the maximal fractional fall and the Day 42 hematocrit, the mean (standard deviation [SD]) absolute loss of RBCs was calculated to be 628,000 cells/ μL (440,000 cells/ μL). The contribution of loss of parasitized RBCs to this figure was estimated by calculating the total number of RBCs parasitized during the infection and dividing this figure by the total number of RBCs lost. This calculation assumes that each parasitized cell was lost and that there was no reticulocyte response before the nadir of hematocrit. We have estimated previously that during the acute phase of falciparum malaria (with a parasite multiplication rate of 10-fold per cycle), $\sim 25\%$ of the overall parasite biomass is sequestered in the microvasculature at any one time, although there is wide variability between people depending on the stage and synchronicity of each infection.¹⁷ Taking into account these cells, the total number of malaria parasites that had occurred in the infection was calculated from the admission parasite count, assuming a previous parasite multiplication rate of 10-fold per cycle. The proportion of the total RBCs that were lost as a direct result of parasitization was estimated to be 7.9% (95% CI, 6.2–9.6%). Thus, even if the majority of parasitized cells were

sequestered at the time of admission, parasitization would still only account for less than one fifth of the loss in RBCs in patients with uncomplicated falciparum malaria.

Hematocrit during follow-up

The mean hematocrit was lowest on Day 7 (mean, 32.8%; 95% CI, 32.6–32.9) (Figure 1). The fractional fall in hematocrit during the first week of treatment correlated positively with hematocrit at admission ($r = 0.6$; $P < 0.001$), and this relationship was independent of the initial parasitemia. In those patients whose hematocrit was $> 30\%$ at admission ($n = 3,280$), the following were found by Cox regression analysis to be independent risk factors associated with the subsequent development of anemia during follow-up: 1) age < 5 years compared with both children aged 5–14 years (AHR, 2.0, 95% CI, 1.5–2.7; $P < 0.001$), and also patients > 14 years old (AHR, 2.6, 95% CI, 1.8–3.6; $P < 0.001$); 2) a prolonged history of illness before admission (> 2 days) (AHR, 1.7, 95% CI, 1.3–2.2; $P < 0.001$); 3) pure *P. falciparum* infections rather than mixed *P. falciparum* and *P. vivax* infections (AHR, 1.8, 95% CI, 1.2–2.6; $P = 0.002$); 4) a palpable liver (AHR, 1.4, 95% CI, 1.0–1.9; $P = 0.05$); 5) female sex (AHR, 1.3, 95% CI, 1.0–1.6; $P = 0.02$); 6) a high parasite count at admission ($> 100,000$ parasites/ μL) (AHR, 1.5, 95% CI, 1.1–2.0; $P = 0.008$); and 7) failure to clear the peripheral parasitemia within 24 hr (AHR, 1.3, 95% CI, 1.0–1.8; $P = 0.03$).

The time taken to return to a normal hematocrit (defined for each age and sex group as a hematocrit above the 25th percentile of the population for Day 42 hematocrit; Table 2) was significantly longer in those patients whose infections recrudesced during the follow-up period ($P = 0.02$). By Day 42, patients who were treated successfully had a significantly higher hematocrit level compared with those whose malaria had recrudesced (36.2%, SD = 3.5% versus 34.7%, SD = 3.9%; $P < 0.001$) (Figure 3). Furthermore, on this day, half (23 of 46, 50%) of patients failing their treatment were anemic (hematocrit $< 30\%$) compared with less than one fifth (235 of 1,264, 19%) of successfully treated patients (age-stratified relative risk, 2.8, 95% CI, 2.0–3.8; $P < 0.001$). These data allow prediction of the effects of falciparum malaria on the overall hematological status of a population in an area with an attack rate of one episode of falciparum malaria per year and a treatment regimen with a 100% cure rate. In these circumstances, the overall mean drop in hematocrit per person would be very small, averaging 0.15% (95% CI, 0.12–0.19) during the year.

Three factors were found independently to delay hematological recovery: 1) anemia at admission (AHR, 1.9, 95% CI, 1.5–2.3; $P < 0.001$); 2) pure *P. falciparum* infections compared with mixed vivax and falciparum malaria (AHR, 1.3, 95% CI, 1.0–1.5; $P = 0.01$); and 3) recrudescence during follow-up (AHR, 1.2, 95% CI, 1.0–1.5; $P = 0.03$).

DISCUSSION

The emergence of multidrug resistance in *P. falciparum* on the western border of Thailand has been rapid. Patients with drug-resistant malaria responded more slowly to their antimalarial treatment, and their infections were more likely to recrudescence later.¹⁸ These 2 factors combined to increase anemia after falciparum malaria. However, several factors have lessened the impact on the community we have studied. The infection rate with *P. falciparum* in the area is low (approximately one infection every 2 years, although nearly all

patients become symptomatic).⁸ Patients also tended to present early to malaria clinics (to which there is ready access) and begin effective antimalarial treatment before high parasite burdens developed. Elsewhere in this area, the population is more dispersed and enjoys less access to appropriate malaria treatment, and in this case, the impact of malaria on anemia would be expected to be greater.

Other factors may have contributed to anemia in this series. The prevalence of hemoglobinopathies is very high in this area of the world. Nondeletional α -thalassemia gene prevalences in Thailand (principally Hb Constant Spring) range 5–15%, and α^0 -thalassemia genes are found in 2.5% of the population in the North.¹⁹ Surveys in this population indicate that 6% of the population has heterozygote beta thalassemia, 2.5% has homozygous hemoglobin E, and 10% of boys or men have mild variant glucose-6-phosphate dehydrogenase (G6PD) deficiency (Weatherall D and Clegg J, unpublished data). Hookworm is also found, but egg burdens are low, and hookworm is not considered a major cause of anemia in this area (unpublished data). With the possible exception of G6PD deficiency, none of these should have contributed to the reversible reduction in hematocrit associated with malaria, although they would have contributed to the background population prevalence of anemia.

The fall in hematocrit attributable to malaria in this study was greatest 1 week after presentation, with a mean reduction of ~ 10% of the RBC mass and fall in hematocrit exceeding 25% in nearly 7% of patients. Approximately one-quarter of the malaria-attributable fall in hematocrit occurred before presentation and three quarters after treatment. The hematological insult was greatest in the youngest age groups, in those with high parasite burdens, and in those presenting with a prolonged history of fever (> 2 days). As in other studies, a delay in the therapeutic response also contributed to an increased risk of malaria-associated anemia.⁵ Malaria induces dyserythropoiesis, and reticulocytosis is depressed in the acute phase of the disease.^{2,20} However, this cannot account for the rapidity with which anemia develops in malaria; the main causative factor is hemolysis.² This is conventionally attributed to loss of parasitized erythrocytes, but there is also evidence of accelerated destruction of unparasitized erythrocytes.¹⁹⁻²²

Recent studies indicate that the spleen can remove young intraerythrocytic malaria parasites and return the once-parasitized RBCs to circulation.²³ Nevertheless, even making the conservative assumption that each parasitized RBC will be destroyed at schizogony, we estimated that > 90% of the RBCs lost were unparasitized in these patients with uncomplicated falciparum malaria. Although fever and the inflammatory reaction may shorten RBC survival, anemia in malaria is considerably greater than in other systemic infections.²⁴ Recent studies indicate that the membrane properties of unparasitized erythrocytes are altered in acute malaria, and there is reduced RBC deformability,²⁵ which correlates with the degree of anemia.²⁶ The splenic threshold for the removal of rigid or antibody-coated RBCs is lowered, and this is the most likely route of erythrocyte removal in acute falciparum malaria.^{27,28}

Anemia can be considered a measure of the cumulative impact of malaria on the individual patient. Protracted infections are associated with clinically significant RBC destruction, and

in this study, patients with a prolonged history of fever (> 2 days) were 1.5-fold more likely to present with anemia and nearly 3 times more likely to experience a decrease in their hematocrit of > 25%. The hematological burden of a heavy parasite load manifested itself mainly after treatment; indeed, patients with high parasite burdens presented with less RBC destruction before treatment compared with those with lower parasite burdens (0.3% compared with 2.7%; $P = 0.02$).

Immunity is slowly acquired with repeated malaria infection and manifests initially by reduced production of proinflammatory cytokines, as well as by a reduced response to these mediators in the face of infection (premunition). Eventually, more specific immunity supervenes, which controls and may prevent infection. Despite the low level of malaria transmission, there is evidence of acquired immunity in this community.⁸ Children are more likely than adults to develop severe malaria²⁹ and are more likely to fail to respond to antimalarial medication.^{16,30} In this study, children were also at greater risk of developing anemia, but this was still apparent after controlling for parasitemia. This suggests that children may be intrinsically more likely to develop anemia in falciparum malaria. Children with severe malaria are also more likely to develop severe anemia than adults.

In this large series, children aged < 5 years were 5.5 (95% CI, 3–10) times more likely to present with severe anemia (hematocrit < 20%) at admission ($P < 0.001$) and, after controlling for confounding factors, were twice as likely to experience a substantial reduction (> 25%) in their hematocrit when compared with older patients. Factors unrelated to falciparum malaria, such as previous malaria and hookworm infections (both of which were more common in children), may compound the effects of malaria and predispose the children to a greater hematological insult after infection with *P. falciparum*.

Simultaneous coinfection with *P. vivax* attenuated the anemia resulting from falciparum malaria. The mean (SD) hematocrit on Day 7 was 32.5% (4.7) for pure falciparum infections compared with 33.5% (3.9) for mixed infections ($P < 0.001$). In a recent study of the treatment of pure *P. vivax* infections at the same study site, the hematocrit on Day 7 after vivax malaria was found to be 35.6% (3.8).³¹ In the study presented here, patients with mixed infections were 1.8 (95% CI: 1.2–2.6) times less likely to develop anemia during follow-up, and cleared their anemia 1.3 (95% CI: 1.0–1.5) times faster when compared with patients with pure *P. falciparum* infections ($P < 0.001$). This may reflect mutual suppression of the 2 infections or earlier presentation and treatment, perhaps because of the lower pyrogenic density of *P. vivax*.

In this community, mixed infections are also associated with a 4-fold lower risk of developing severe malaria⁸ and of recrudescence after antimalarial treatment.¹⁶ These observations further support the contention that *P. vivax* ameliorates falciparum malaria,³² and they raise important questions over the value of targeting *P. vivax*, either with chloroquine or through the development of a vaccine, in areas where both species are prevalent.

The fractional drop in hematocrit in the first week of treatment was correlated with the baseline hematocrit ($r_s = 0.6$, $P < 0.001$), and this was independent of initial parasitemia.

Hence, patients presenting with a low hematocrit are proportionally less likely to experience a drop in their hematocrit levels than those with high initial hematocrits. This suggests that RBC loss in an effectively treated uncomplicated infection is relatively fixed. Hematological recovery after an acute episode of uncomplicated falciparum malaria was complete by Day 42 of follow-up. After this time, hematocrit values did not change in those who were cured, and age-stratified values were not significantly different from those obtained from a hematological survey of the population. This period of recovery is twice that observed in healthy adults after a comparable loss of blood volume (8%).³³ This slow recovery may result from dyserythropoiesis and continued destruction of unparasitized erythrocytes after clearance of parasitemia.²¹ Patients whose infections recrudesced during follow-up took significantly longer to return to a normal hematocrit, and by Day 42, these patients were nearly 3 times as likely to be anemic ($P < 0.001$).

In the majority of patients, a single episode of uncomplicated falciparum malaria, which is diagnosed and treated effectively, causes mild anemia; this resolves slowly. Drug resistance leads to an increase in anemia, and young children are particularly vulnerable because they are more likely to have recrudescing infections and have a greater decrease in hematocrit levels than older children for any given severity of infection.

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REFERENCES

1. Hedberg K, Shaffer N, Davachi F, Hightower A, Lyamba B, Paluku KM, Nguyen Dinh P, Breman JG. *Plasmodium falciparum*-associated anemia in children at a large urban hospital in Zaire. *Am J Trop Med Hyg.* 1993; 48:365–371. [PubMed: 8470774]
2. White, NJ.; Ho, M. The pathophysiology of malaria. In: Baker, JR.; Muller, R., editors. *Advances in Parasitology.* Academic Press; New York: 1992. p. 84-175.
3. Slutsker L, Taylor TE, Wirima JJ, Steketee RW. In-hospital morbidity and mortality due to malaria-associated severe anaemia in two areas of Malawi with different patterns of malaria infection. *Trans R Soc Trop Med Hyg.* 1994; 88:548–551. [PubMed: 7992335]
4. Ekvall H, Premji Z, Bjorkman A. Chloroquine treatment for uncomplicated childhood malaria in an area with drug resistance: early treatment failure aggravates anaemia. *Trans R Soc Trop Med Hyg.* 1998; 92:556–560. [PubMed: 9861379]
5. Bloland PB, Lackritz EM, Kazembe PN, Were JB, Steketee R, Campbell CC. Beyond chloroquine: implications of drug resistance for evaluating malaria therapy efficacy and treatment policy in Africa. *J Infect Dis.* 1993; 167:932–937. [PubMed: 8450258]
6. Zucker JR, Lackritz EM, Ruebush TK, Hightower AW, Adungosi JE, Were JB, Campbell CC. Anaemia, blood transfusion practices, HIV and mortality among women of reproductive age in western Kenya. *Trans R Soc Trop Med Hyg.* 1994; 88:173–176. [PubMed: 8036663]
7. Zucker JR, Lackritz EM, Ruebush TK, Hightower AW, Adungosi JE, Were JBO, Metchock B, Patrick E, Campbell CC. Childhood mortality during and after hospitalisation in Western Kenya: effect of malaria treatment regimens. *Am J Trop Med Hyg.* 1997; 55:655–660. [PubMed: 9025694]

8. Luxemburger C, Ley Kyaw Thew, White NJ, Webster HK, Kyle DE, Maelankiri L, Chongsuphajaisiddhi T, Nosten F. The epidemiology of malaria in a Karen population on the western border of Thailand. *Trans R Soc Trop Med Hyg.* 1996; 90:105–111. [PubMed: 8761562]
9. ter Kuile FO, Dolan G, Nosten F, Edstein MD, Luxemburger C, Phaipun L, Chongsuphajaisiddhi T, Webster HK, White NJ. Halofantrine versus mefloquine in the treatment of multi-drug resistant falciparum malaria. *Lancet.* 1993; 341:1044–1049. [PubMed: 8096956]
10. Luxemburger C, ter Kuile F, Nosten F, Dolan G, Bradol JH, Phaipun L, Chongsuphajaisiddhi T, White NJ. Single day mefloquine-artesunate combination in the treatment of multi-drug resistant falciparum malaria. *Trans R Soc Trop Med Hyg.* 1994; 88:213–217. [PubMed: 8036679]
11. Nosten F, Luxemburger C, ter Kuile FO, Woodrow C, Eh JP, Chongsuphajaisiddhi T, White NJ. Treatment of multi-drug-resistant *Plasmodium falciparum* malaria with 3-day artesunate-mefloquine combination. *J Infect Dis.* 1994; 170:971–977. [PubMed: 7930743]
12. Luxemburger C, Nosten F, Raimond SD, Chongsuphajaisiddhi T, White NJ. Oral artesunate in the treatment of uncomplicated hyperparasitemic falciparum malaria. *Am J Trop Med Hyg.* 1995; 53:522–525. [PubMed: 7485711]
13. Price RN, Nosten F, Luxemburger C, Am Kham, Brockman A, Chongsuphajaisiddhi T, White NJ. Artesunate versus artemether in combination with mefloquine for the treatment of multidrug resistant falciparum malaria. *Trans R Soc Trop Med Hyg.* 1995; 89:523–527. [PubMed: 8560531]
14. Price RN, Luxemburger C, van Vugt M, Simpson J, Phaipun L, Chongsuphajaisiddhi T, Nosten F, White NJ. Treatment of uncomplicated hyperparasitaemic falciparum malaria on the Thai-Burmese border. *Trans R Soc Trop Med Hyg.* 1998; 92:207–211. [PubMed: 9764335]
15. Price RN, van Vugt M, Luxemburger C, Brockman A, Phaipun L, Chongsuphajaisiddhi T, Nosten F, White NJ. Artesunate versus artemether for the treatment of recrudescing falciparum malaria. *Am J Trop Med Hyg.* 1998; 59:883–888. [PubMed: 9886194]
16. Price RN, Nosten F, Luxemburger C, van Vugt M, Phaipun L, Chongsuphajaisiddhi T, White NJ. Artesunate/mefloquine treatment of multi-drug resistant falciparum malaria. *Trans R Soc Trop Med Hyg.* 1997; 91:574–577. [PubMed: 9463672]
17. White NJ, Chapman D, Watt G. The effects of multiplication and synchronicity on the vascular distribution of parasites in falciparum malaria. *Trans R Soc Trop Med Hyg.* 1992; 86:590–597. [PubMed: 1287908]
18. White NJ. Assessment of the pharmacodynamic properties of antimalarial drugs in vivo. *Antimicrob Agents Chemother.* 1997; 41:1413–1422. [PubMed: 9210658]
19. Hundreiser J, Sanguansermsri T, Papp T, Flatz G. Alpha-thalassaemia in Northern Thailand. *Hum Hered.* 1988; 38:211–215. [PubMed: 3169795]
20. Phillips RE, Looareesuwan S, Warrell DA, Lee SH, Karbwang J, Warrell MJ, White NJ, Swasdichai C, Weatherall DJ. The importance of anaemia in cerebral and uncomplicated falciparum malaria: role of complications, dyserythropoiesis and iron sequestration. *Q J Med.* 1986; 58:305–323. [PubMed: 3526385]
21. Looareesuwan S, Merry AH, Phillips RE, Pleehachinda R, Wattanagoon Y, Ho M, Charoenlarp P, Warrell DA, Weatherall DJ. Reduced erythrocyte survival following clearance of malarial parasitemia in Thai patients. *Br J Haematol.* 1987; 67:473–478. [PubMed: 3322362]
22. Looareesuwan S, Davis TME, Pukrittayakamee S, Supanaranond W, Desakorn V, Silamut K, Krishna S, Boonamrung S, White NJ. Erythrocyte survival in severe falciparum malaria. *Acta Trop.* 1991; 48:263–270. [PubMed: 1674400]
23. Angus B, Chotivanich K, Udomsangpetch R, White NJ. In vivo removal of malaria parasites from red cells without their destruction in acute falciparum malaria. *Blood.* 1997; 90:2037–2040. [PubMed: 9292540]
24. Karle H. The pathogenesis of the anaemia of chronic disorders and the role of fever in erythrokinetics. *Scand J Haematol.* 1994; 13:81–86. [PubMed: 4608900]
25. Naumann KM, Jones GL, Saul A, Smith R. Parasite-induced changes to localized erythrocyte membrane deformability in *Plasmodium falciparum* cultures. *Immun Cell Biol.* 1992; 70:267–275.
26. Dondorp AM, Angus BJ, Vreeken J, Chotivanich K, Silamut K, Ruangveerayuth R, Hardeman MR, Kager PA, White NJ. Red cell deformability as a predictor of anemia in severe falciparum malaria. *Am J Trop Med Hyg.* 1999; 60:733–737. [PubMed: 10344643]

27. Looareesuwan S, Ho M, Wattanagoon Y, White NJ, Warrell DA, Bunnag D, Harinasuta T, Wyler DJ. Dynamic alteration in splenic function during acute falciparum malaria. *N Engl J Med.* 1987; 317:675–679. [PubMed: 3306376]
28. Ho M, White NJ, Looareesuwan S, Wattanagoon Y, Lee SH, Walport MJ, Bunnag D, Harinasuta T. Splenic Fc receptor function in host defense and anemia in acute *Plasmodium falciparum* malaria. *J Infect Dis.* 1990; 161:555–561. [PubMed: 2179426]
29. Luxemburger C, Ricci F, Nosten F, Raimond D, Bathet S, White NJ. The epidemiology of severe malaria in an area of low transmission in Thailand. *Trans R Soc Trop Med Hyg.* 1997; 91:256–262. [PubMed: 9231189]
30. ter Kuile F, Luxemburger C, Nosten F, Phaipun L, Chongsuphajaisiddhi T, White NJ. Predictors of mefloquine treatment failure: a prospective study in 1590 patients with uncomplicated falciparum malaria. *Trans R Soc Trop Med Hyg.* 1995; 89:660–664. [PubMed: 8594690]
31. Luxemburger C, van Vugt M, Jonathan S, McGready R, Looareesuwan S, White NJ, Nosten F. Treatment of vivax malaria on the western border of Thailand. *Trans R Soc Trop Med Hyg.* 1999; 93:433–438. [PubMed: 10674098]
32. Maitland K, Williams TN, Newbold CI. *Plasmodium vivax* and *P. falciparum*: biological interactions and the possibility of cross-species immunity. *Parasitol Today.* 1997; 13:227–231. [PubMed: 15275075]
33. Wadsworth GB. Recovery from acute haemorrhage in normal men and women. *J Physiol.* 1955; 129:583–591. [PubMed: 13264119]

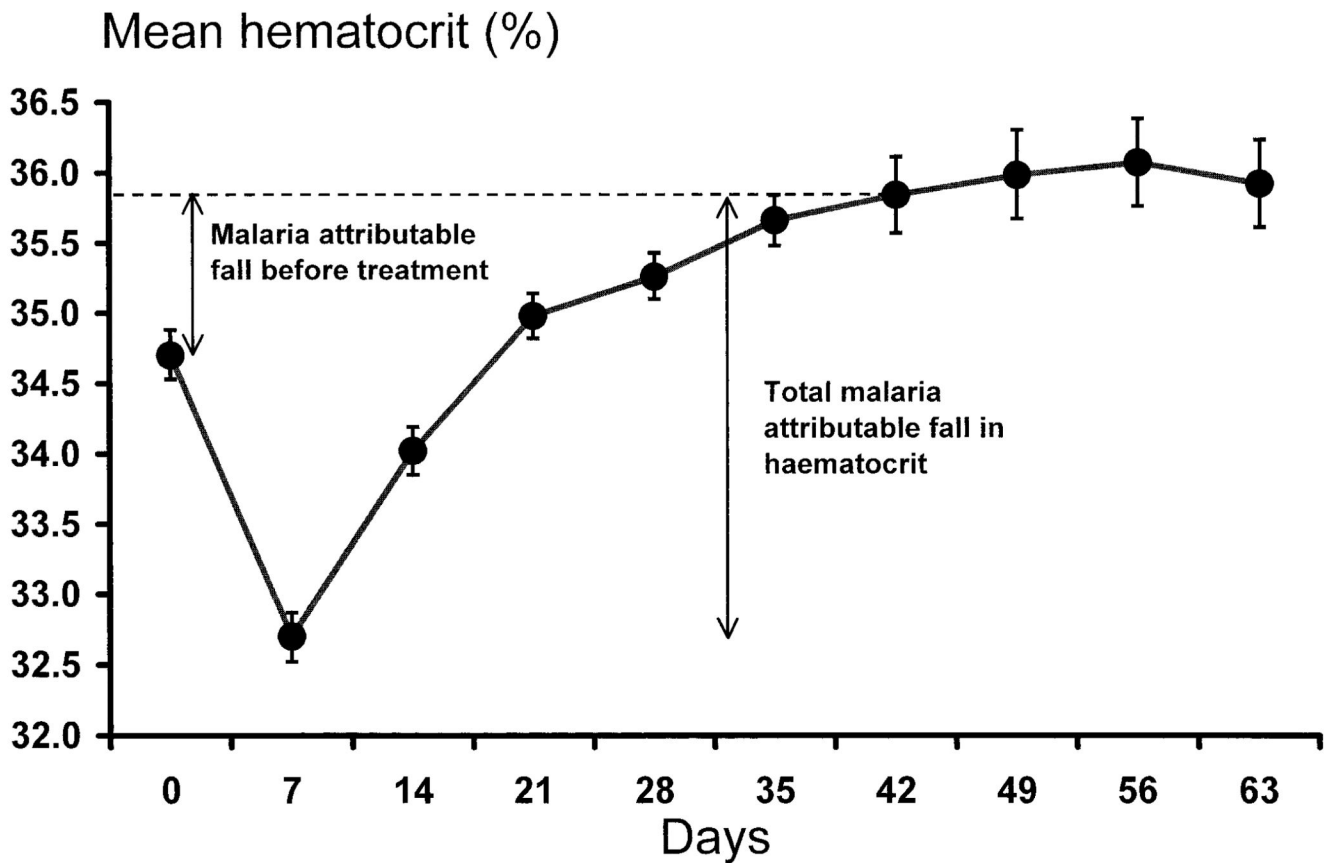


Figure 1.

Hematological recovery after an episode of falciparum malaria (mean and 95% confidence intervals) in 4,007 patients. The malaria attributable fall in hematocrit before and after treatment was defined as the difference between the patients' hematocrit on Day 42 and that on Day 0 or Day 7, respectively.

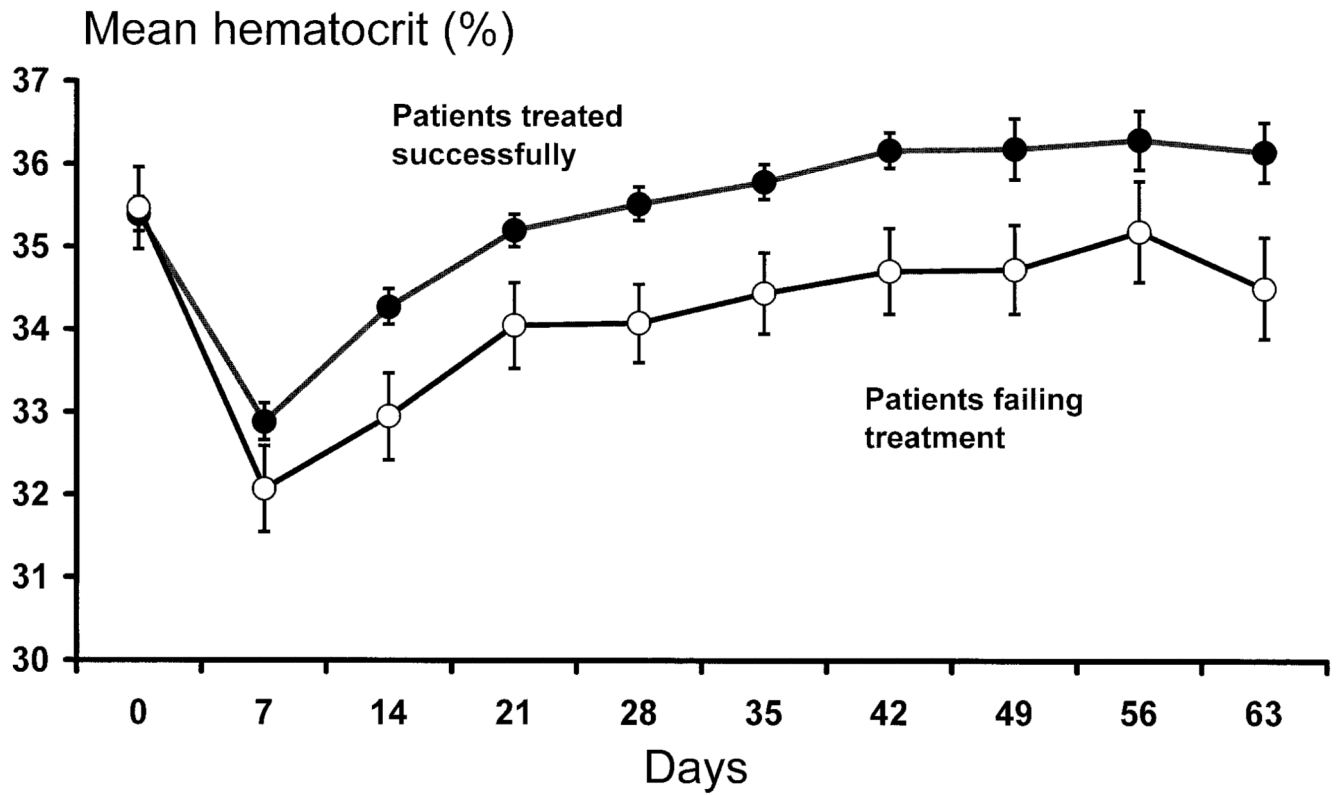


Figure 2.
The age distribution of the mean maximal fractional fall in hematocrit associated with malaria.

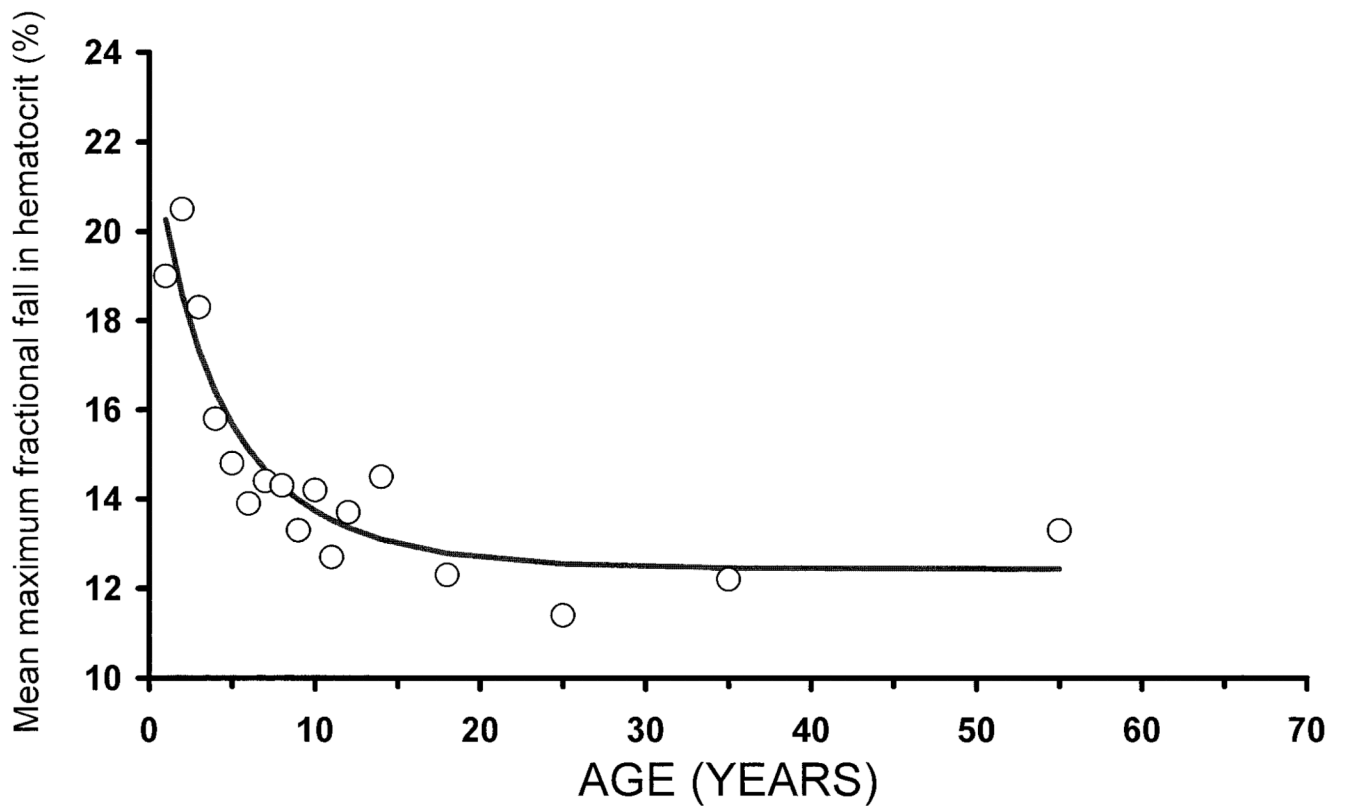


Figure 3. Hematological recovery after a primary episode of falciparum malaria (mean and 95% confidence intervals) for patients treated successfully and patients with recrudescent infections during follow-up ($n = 3,021$).

Table 1

Antimalarial treatment regimens

| Drug and regimen | <i>n</i> | Dose |
|--------------------------------------|----------|--|
| Mefloquine | | |
| M ₂₅ | 1,324 | 25 mg/kg |
| Halofantrine | | |
| H ₂₄ | 106 | 24 mg/kg in 1 day |
| H ₇₂ | 357 | 72 mg/kg over 3 days |
| Artesunate + mefloquine | | |
| MA | 319 | As 10 mg/kg (3 doses in 1 day) + M15 |
| MAS1 | 150 | As 4 mg/kg (1 dose) + M25 |
| MAS3 | 1,729 | As 4 mg/kg daily for 3 days + M25 |
| MAS5 | 44 | As 12 mg/kg (over 5 days) + M25 |
| MAS7 | 97 | As 12 mg/kg (over 7 days) + M25 |
| Artesunate | | |
| AS5 | 148 | 12 mg/kg over 5 days |
| AS7 | 401 | 12 mg/kg over 7 days |
| Artemether | | |
| MAM3 | 201 | Am 4 mg/kg daily for 3 days + M25 |
| AM7 | 157 | 12 mg/kg over 7 days |
| Quinine with or without tetracycline | | |
| Q7 | 38 | Q 30 mg/kg daily for 7 days |
| Q7T7 | 182 | Q 30 mg/kg daily for 7 days + T 16 mg/kg daily |

As = Artesunate, Am = Artemether, M15 = Mefloquine 15 mg/kg, M25 = Mefloquine 25 mg/kg, Q = Quinine, T = Tetracycline.

Table 2

Comparison of the hematocrit values of patients at the time of complete hematological recovery* and from a survey of the general population conducted during the study period

| Age and sex | Survey | | 42 days after acute malaria | | |
|-------------|----------|---|-----------------------------|---|-----------------|
| | <i>n</i> | Mean hematocrit, % (standard deviation) | <i>n</i> | Mean hematocrit, % (standard deviation) | 25th percentile |
| Total | 311 | 36.0 (4.4) | 2,151 | 36.0 (3.2) | 34.0 |
| <5 years | 56 | 33.8 (3.8) | 360 | 34.4 (3.1) | 33.0 |
| 5–14 years | 123 | 36.5 (4.1) | 1,123 | 36.1 (2.9) | 34.5 |
| Men | 44 | 38.7 (4.7) | 324 | 38.1 (3.5) | 36.0 |
| Women | 88 | 35.3 (4.8) | 344 | 35.6 (2.9) | 34.0 |

* Derived from the predicted and actual hematocrit values on Day 42 of follow-up.

Table 3

Admission characteristics

| Variable | Patients with admission hematocrit, <i>n</i> (%) | Admission hematocrit, mean (standard deviation) | Anemic (hematocrit < 30), <i>n</i> (%) | Severe anemia (hematocrit < 20), <i>n</i> (%) |
|--|--|---|--|---|
| Total | 4,007 (76) | 34.7 (5.7) | 727 (18) | 40 (1.0) |
| Aged < 5 years | 613 (71) | 31.1 (5.7) | 235 (38) | 20 (3.3) |
| Aged 5–14 years | 1,905 (79) | 34.9 (5.2) | 304 (16) | 16 (0.8) |
| Men | 786 (75) | 37.4 (5.6) | 63 (8) | 1 (0.1) |
| Women | 703 (76) | 34.3 (5.1) | 125 (18) | 3 (0.4) |
| Primary infections | 3,046 (79) | 35.3 (5.6) | 476 (16) | 25 (0.8) |
| Recrudescence infections | 961 (68) | 33.0 (5.5) | 251 (26) | 15 (1.6) |
| <i>Plasmodium falciparum</i> only | 3,328 (77) | 34.7 (5.9) | 630 (19) | 35 (1.1) |
| <i>P. falciparum</i> and <i>Plasmodium vivax</i> | 634 (74) | 35.2 (5.1) | 88 (14) | 4 (0.6) |

Table 4

Population attributable risk for a fractional fall in hematocrit of > 25% before and after malaria treatment

| Risk factor | Frequency (%) | AOR (95% confidence interval)* | PAR [†] (%) | P value |
|--|---------------|--------------------------------|----------------------|---------|
| Before treatment [‡] | | | | |
| Aged 5 years | 17 | 2.6 (2.2–3.1) | 21.4 | <0.001 |
| Recrudescence infections | 27 | 1.9 (1.5–2.4) | 19.5 | 0.005 |
| Palpable spleen | 23 | 2.7 (2.3–3.2) | 28.1 | <0.001 |
| Palpable liver | 16 | 1.8 (1.4–2.3) | 11.3 | 0.01 |
| After treatment [§] | | | | |
| Aged 5 years | 17 | 2.2 (1.7–2.7) | 16.9 | <0.001 |
| High parasitemia (μ 100,000 parasites/ μ L) | 10 | 2.4 (1.9–2.9) | 12.3 | <0.001 |
| Prolonged preadmission history | 65 | 2.9 (2.4–3.5) | 55.3 | <0.001 |
| Parasitemia not cleared in 24 hr | 64 | 2.7 (2.1–3.2) | 52.1 | <0.001 |

* AOR = adjusted odds ratio. AORs and 95% confidence intervals of the risk associated with the presence of the factor when compared with its absence after adjustment for the other factors in the table. AORs were calculated by logistic regression analysis.

[†] PAR = population attributable risk. PAR is for a fractional drop in hematocrit from normal values of > 25%. The overall PAR was calculated as 1 minus the product of 1 minus each individual PAR.

[‡] Overall PAR before treatment is 59.6.

[§] Overall PAR after treatment is 84.4.