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Therapeutic Efficacy and Effects of Artesunate-Mefloquine and Mefloquine Alone on Malaria-Associated Anemia in Children with Uncomplicated *Plasmodium falciparum* Malaria in Southwest Nigeria

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Abstract. The treatment efficacy and effects of artesunate-mefloquine (AMQ) and mefloquine (MQ) on malaria-associated anemia (MAA) were evaluated in 342 children ≤ 10 years of age with uncomplicated *Plasmodium falciparum* malaria randomized to receive either drug/drug combination. All children recovered clinically. Fever clearance times were similar. Parasite clearance was significantly faster with AMQ (mean ± SD = 1.4 ± 0.6 days, 95% confidence interval [CI] = 1.3–1.5, $P < 0.0001$), but polymerase chain reaction–corrected cure rates were similar (97% versus 94%). Gametocyte carriage rates and the drug-attributable fall in hematocrit were significantly lower with AMQ (mean ± SD = 4.8 ± 3.8%, 95% CI = 3.6–6.0, $P = 0.03$), but the rates of resolution of MAA were similar. Both regimens were well tolerated. AMQ clears parasitemia and reduces gametocyte carriage more rapidly and causes lesser fall in hematocrit than MQ, but both regimens are effective treatment of uncomplicated *P. falciparum* malaria in Nigerian children.

INTRODUCTION

The development and spread of drug resistance in *Plasmodium falciparum* to many antimalarials is of global concern and a major public health problem in malaria-endemic countries in Africa.[1–4] As part of the strategy to combat the spread of antimalarial drug resistance globally, the World Health Organization recommended the use of artemisinin-based combination therapies (ACTs) because they quickly reduce the burden of parasitemia and gametocytemia and the chance of drug resistance.[1,5–9] Artesunate-mefloquine (AMQ) combination is not considered a viable option for use as first-line therapy in Africa because of intense transmission.[2] However AMQ, co-packaged as well as fixed dose formulations, is readily available and used in Africa. Despite high efficacy[9,10] and increasing use of ACTs in Africa,[11] many individuals still use antimalarial monotherapies because they are inexpensive and readily available, and the parasite has remained largely sensitive to, for example, mefloquine (MQ), despite the reported innate resistance to this drug in some areas of West Africa.[12,13] Additionally, the inverse relationship in the sensitivity of *P. falciparum* to MQ and chloroquine (CQ) in West Africa[14] has further renewed interest in the use of MQ, because high-grade CQ resistance is common in the sub-region.

Virtually all of the studies comparing AMQ with MQ have been conducted in areas of low transmission.[15] There is no reported study of the efficacy of AMQ versus MQ in African children living in areas of intense transmission despite the availability and ready use of artesunate and MQ in these areas. It is also unclear whether MQ-based ACTs have superior efficacy to MQ alone in Africa, making it imperative to evaluate such regimens in sub-Saharan Africa. Such a study is essential as it may, in the future, influence policy and management of drug resistance in the community.

In this study, we report the tolerability, antimalarial treatment efficacy, effects on gametocyte carriage, and malaria-associated anemia of AMQ and MQ in children ≤ 10 years of age with acute, symptomatic, uncomplicated, *P. falciparum* malaria.

MATERIALS AND METHODS

Study area. The study was carried out in Ibadan, southwest Nigeria, from July 2007 to August 2008. In this area of hyperendemic malaria, transmission occurs all year round but is more intense during the rainy season from April to October.[16] *In vitro* and *in vivo* MQ resistance of 14% and 0%, respectively, and *in vitro* reduced susceptibility to artemisinin (the parent drug from which artesunate is derived) in 5% of *P. falciparum* isolates in the area in the 1990s have been reported,[12–14,17] but there are no current estimates of failure rates.

Patients, treatment, and follow-up. Patients were eligible to join the study if they were ≤ 10 years of age, had symptoms compatible with acute uncomplicated malaria such as anorexia, vomiting, or abdominal discomfort with or without diarrhea, with *P. falciparum* parasitemia > 2,000 asexual forms/μL, a body (axillary) temperature > 37.4°C or history of fever in the 24–48 hours preceding presentation, absence of other concomitant illness, no history of antimalarial use in the 2 weeks preceding presentation, negative urine tests for antimalarial drugs (Dill-Glazko and lignin for 4-aminoquinolines and sulfonamides, respectively),[18,19] and written informed consent given by parents or guardians. Patients with severe malaria,[20] severe malnutrition, serious underlying diseases (renal, cardiac, or hepatic), and known allergy to study drugs were excluded from the study. The study protocol was reviewed and approved by the Ethics Committee of The Ministry of Health, Ibadan. The disease history, taken by the attending physician, was recorded by asking patients or their parents/guardians when the present symptomatic period started and was followed by a full physical examination by the same physician.

Enrolled patients were randomly assigned to receive AMQ or MQ. AMQ or MQ alone was given according to body weight: artesunate at a dose of 4 mg/kg daily at presentation (Day 0) and daily for a further 2 days (Days 1 and 2) and MQ at a dose of 25 mg/kg at presentation only.

All drugs were given orally as direct observed therapy by the physician. After drug administration, all patients waited

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for at least 3 hours after to ensure the drug was not vomited. If it was, the patient was excluded from the study. If necessary, patients were provided with antipyrectics (paracetamol tablets, 10–15 mg/kg every 8 hours for 24 hours). The randomization was computer generated, and treatment codes were sealed in individual envelopes. Patient evaluation and follow-up after drug administration was performed by another physician blinded to the drug treatment. The study nurse obtained thick and thin blood films from each child as soon as they came to the clinic. The slides were carefully labeled with the patients' codes and were air dried before being stained.

Follow-up with clinical and parasitologic evaluation was done daily on Days 1–7 and then on Days 14, 21, 28, 35, and 42. This consisted of enquiry about the patient's well being, presence or absence of initial presenting symptoms, presence of additional symptoms, measurement of body temperature, heart and respiratory rates, and taking a blood smear for the quantification of parasitemia.

Side effects were defined as symptoms and signs that first occurred or became worse after treatment was started. Any new events occurring during treatment were also considered as side effects.

Thick and thin blood films prepared from a finger prick were stained with Giemsa and were examined by light microscopy under an oil-immersion objective, at ×1,000 magnification, by two independent assessors who did not know the drug treatment of the patient. A senior member of the study team reviewed the slides if there was any disagreement between the microscopists. In addition, the slides of every third child enrolled in the study were reviewed by this senior member. Parasitemia (asexual or sexual) in thick films was estimated by counting asexual or sexual parasites relative to 1,000 leukocytes, or 500 asexual or sexual forms, whichever occurred first. From this figure, the parasite density was calculated assuming a leukocyte count of 6,000/μL of blood.

Capillary blood collected before and during follow-up was used to measure packed cell volume (PCV). PCVs were measured using a microhematocrit tube and microcentrifuge (Hawksley, Lancing, UK). Drug-attributable fall in hematocrit (DAFH) during treatment was defined as the difference between patient's hematocrit on Day 0 and Day 3 after starting treatment.

Blood was spotted on filter papers on Days 0, 3, 7, 14, 21, 28, 35, and 42 and at the time of treatment failures for parasite genotyping. Paired primary and post-treatment parasites were analyzed using parasite loci that exhibit repeated numbers of polymorphisms to distinguish true treatment failures from new infections. Briefly, Block 2 of merozoite surface protein-1 (MSP-1) and Block 3 of merozoite surface protein-2 (MSP-2) and region II of glutamine-rich protein (GLURP) were amplified by two rounds of polymerase chain reaction (PCR) using primers and amplification conditions described previously.[21–24] Ten microliters of the nested PCR products was resolved by electrophoresis on a 2% agarose gel and sized against a 100-bp molecular weight marker (New England Biolabs, Beverly, MA). The banding pattern of the post-treatment parasites was compared with matched primary parasites in each of the patients who had parasitemia after treatment with either artesunate-mefloquine or mefloquine. Post-treatment and primary infection parasites showing identical bands were considered as true treatment failure, whereas non-identity in banding patterns was considered as newly acquired infections.

Response to drug treatment was assessed using WHO 1973 criteria[25] as follows: S, sensitive, clearance of parasitemia without recurrence; RI (mild resistance), parasitemia disappears but reappears within 7–14 days; RII (moderate resistance), decrease of parasitemia but no complete clearance from peripheral blood; RIII (severe resistance), no pronounced decrease or increase in parasitemia at 48 hours after treatment. In those with sensitive or mild resistance, parasite clearance time was defined as the time elapsing between drug administration and absence of detectable parasitemia for at least 48 hours. Times taken to clear 50% and 90% parasitemia were calculated from the plot of decline in parasitemia versus time. Fever clearance time was defined as the time from drug administration until the body temperature fell to or < 37.4°C and remained so for 48 hours. Response to drug treatment was also classified according to a modified version of the WHO 14-day *in vivo* clinical classification system[26]; because all patients were not febrile at enrollment, a temperature < 37.5°C was not an exclusion criterion for enrollment. The modification also involved a follow up for 42 days in this area of intense transmission. The clinical classification system consisted of the following categories of response: adequate clinical and parasitologic response (ACPR), late parasitologic failure (LPF), late clinical failure (LCF), and early treatment failure (ETF).

Cure rates were defined as the percentages of patients whose asexual parasitemia cleared from peripheral blood and who were free of patent asexual parasitemia on Days 14, 21, 28, 35, and 42 of follow-up. The cure rates on Days 21–42 were adjusted on the basis of the PCR genotyping results of paired samples for patients with recurrent parasitemia after Day 14 of starting treatment.

Re-treatment of drug treatment failures. All patients failing treatment (within 35 days) with artesunate-mefloquine or mefloquine were retreated with these drug/drug combination and were followed for 63 days. Patients were re-treated whenever they became symptomatic (usually between 18 and 35 days after initial enrollment). Patients with profound clinical (hyperpyrexia, oral fluid intolerance) and parasitologic deterioration (> 20% increase in baseline parasitemia) during follow-up were treated with parenteral quinine and were regarded as treatment failures.

Data analysis. Sample size was calculated so that the study would be able to detect a 14% absolute difference in parasitologic failure "rate" between AMQ and MQ groups, with 99% power and at a 5% significance level. The expected treatment success rates were 99% for AMQ and 85% for MQ on Days 28–42. The sample size was 147 patients in each treatment arm. Data were analyzed using version 6 of the Epi-Info software[27] and the statistical program SPSS for Windows version 10.01.[28] Variables considered in the analysis were related to the densities of *P. falciparum* gametocytes and trophozoites. Proportions were compared by calculating χ^2 with Yates correction or by Fisher exact or Mantel Haenszel tests. Normally distributed, continuous data were compared by Student *t* tests and analysis of variance (ANOVA). Data not conforming to a normal distribution were compared by the Mann-Whitney *U* tests and the Kruskal-Wallis tests (or by Wilcoxon ranked sum test). All tests of significance, except where specifically indicated, were two-tailed. $P < 0.05$ was taken to indicate significant differences. Data were

(double)-entered serially using the patients' codes and were only analyzed at the end of the study.

RESULTS

Patient characteristics. Between July 2007 and August 2008, 355 patients were recruited: 175 in the AMQ group and 180 in the MQ group (Figure 1). There were 102 children < 5 years old: 56 (32%) in the AMQ group and 46 (25%) in the MQ group. Overall, 328 patients (166 in the AMQ group and 162 in the MQ group) completed 42 days of follow-up; 13 children, 4 in AMQ and 9 in MQ, were excluded because of failure to meet all inclusion criteria. Three hundred forty-two children completed at least 21 days of follow-up. Twelve children, five in the AMQ group and seven in the MQ group, could not be evaluated because of protocol violation or relocation from study area. Overall results are for 342 children. The baseline characteristics were similar for both treatment groups (Table 1). However, mean hematocrit value was significantly lower in children enrolled in the AMQ treatment arm.

Fever and parasite clearance. Two hundred thirty-seven children were febrile at enrollment: 120 in the AMQ group and 117 in the MQ group. On the whole, 120 of the children treated with AMQ and 117 of those treated with MQ received paracetamol during the first 24 hours. By Day 1, fever cleared in 99 and 94 children, respectively. There was no significant difference in the proportion of patients in whom fever cleared by Day 1 ($\chi^2 = 0.07$, $P = 0.8$). By Day 2, 6 and 16 children were still febrile in the AMQ and MQ groups, respectively ($\chi^2 = 4.3$, $P = 0.03$). However, overall, fever clearance was similar in the two treatment groups (Table 2).

Compared with MQ, AMQ substantially accelerated the clearance of parasitemia. By Day 1, 11 children in the AMQ treatment arm and 67 children in the MQ treatment arm had not cleared their parasitemias. The difference in this proportion was significant ($\chi^2 = 50.2$, $P < 0.0001$). Times to clear 50%

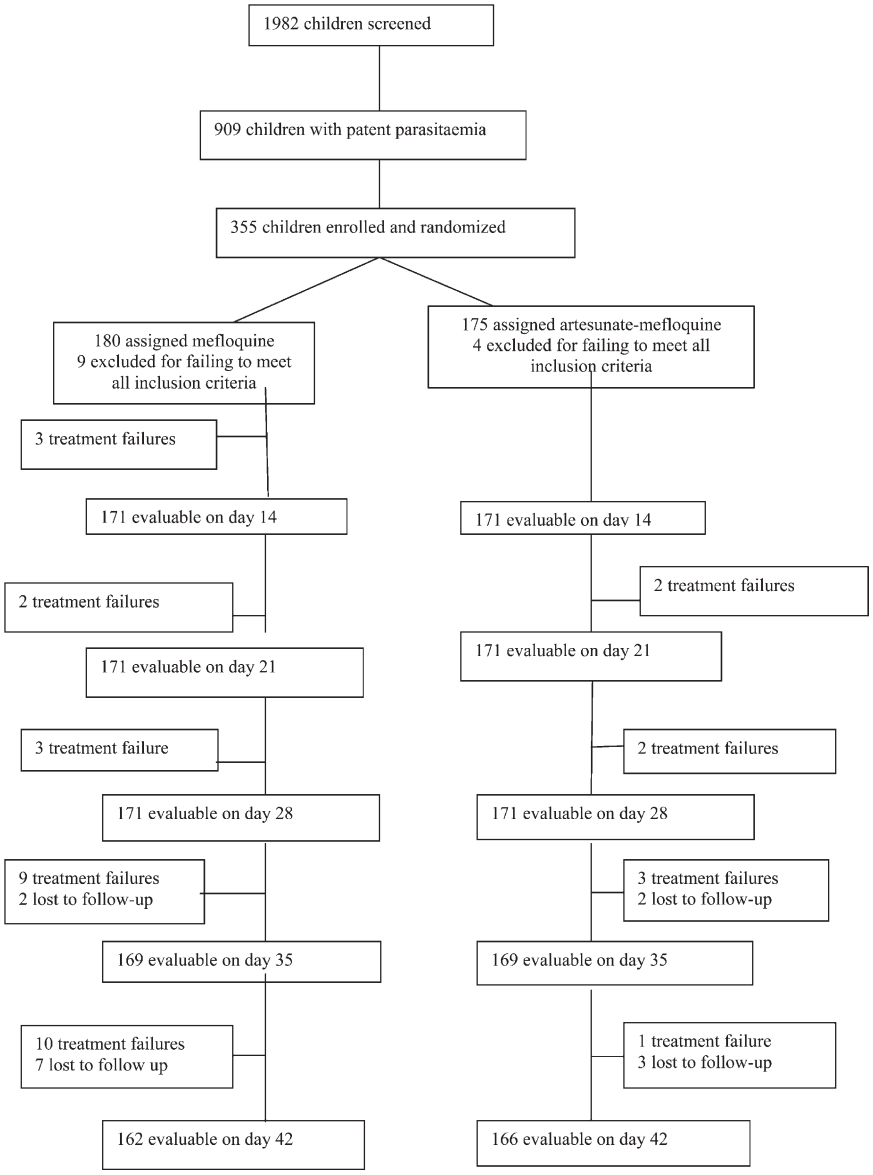

FIGURE 1. Trial profile.

Table 1
Demographic and clinical characteristics of patients at enrollment

Variable	AMQ	MQ	*P* value
No. of patients	171	171	–
Male/female	83/88	90/81	–
Age (months)			
Mean ± SD	74.4 ± 34.8	80.4 ± 34.8	0.11
Range	6–120	7–120	
Number < 5 years	56	46	0.28
Weight (kg)			
Mean ± SD	18.5 ± 6.5	19.7 ± 6.1	0.07
Range	7–46	7–36	
Duration of illness (days)			
Mean ± SD	2.9 ± 1.1	2.9 ± 1.1	1.0
Range	1–7	1–10	
Temperature (°C)			
Mean ± SD	38.3 ± 1.1	38.0 ± 1.8	0.06
Range	36.2–40.9	36.2–40.6	
Number > 40°C	11	10	1.0
Parasite count (/μL)			
Geometric mean	30,654	30,770	0.9
Range	2,484–884,571	2,400–1,402,635	
Number with > 250,000/μL	9	16	
Gametocyte count (/μL)			
Geometric mean	19 (*N* = 22)	23 (*N* = 20)	0.24
Range	6–216	6–468	
Hematocrit (%)			
Mean ± SD	31.1 ± 5.9 (*N* = 119)	32.8 ± 5.4 (*N* = 126)	0.02
Range	13–48	18–49	
Number < 30%	43	33	0.12

AMQ = artesunate-mefloquine; MQ = mefloquine.

and 90% parasitemia were significantly shorter, and overall, parasite clearance was significantly shorter in those treated with AMQ (1.4 ± 0.6 versus 2.1 ± 1.0 days, $P < 0.0001$; Table 2). No patient received rescue medication.

Response to both treatment regimens was not related to age: 1 of 56 and 7 of 115 < 5 and ≥ 5 year olds, respectively, treated with AMQ failed treatment by Day 28 ($P = 0.27$, Fisher exact test). Similarly, 5 of 46 and 8 of 125 < 5 and ≥ 5 year olds, respectively, treated with MQ failed treatment by Day 28 ($P = 0.33$, by Fisher exact test). All 171 patients treated with AMQ and 168 of 171 patients treated with MQ had adequate clinical and parasitologic response (ACPR) up to Day 14.

Gametocyte carriage. Gametocytes were detected in the peripheral blood in 108 children (31%) from the two treatment groups (Table 3). The overall detection rate at enrollment was 12% and was not significantly different between the two treatment groups ($P = 0.71$). After treatment, the emergence of gametocytes was significantly less frequent in the artesunate-mefloquine group than in the mefloquine alone group ($P = 0.004$; Table 3). Post-treatment, gametocyte carriage was significantly higher than pre-treatment in the mefloquine alone group ($P = 0.001$) but not in the artesunate-mefloquine group ($P = 0.87$; Table 3). Analysis of daily gametocyte carriage rates showed that carriage was significantly lower from Days 2 to 7 in those treated with AMQ compared with those treated with MQ ($P < 0.03$ at all times).

Rate of re-appearance of parasitemia. There were 28 patients, 9 in the AMQ group and 19 in the MQ treatment group, in whom, after initial clearance, parasitemia reappeared between Days 7 or 14 and 42 (Table 2). The cumulative proportion of children with *P. falciparum* reappearance

Table 2
Therapeutic responses to artesunate-mefloquine or mefloquine

	AMQ	MQ	*P* value
No. of patients	171	171	–
Fever clearance time (d)			
Mean ± SD	1.2 ± 0.6	1.3 ± 0.7	0.23
Range	1–5	1–4	
95% CI	1.1–1.4	1.2–1.4	
No. of patient with parasitemia on day 1	63	104	< 0.0001
No. of patient with parasitemia on day 2	11	36	< 0.0001
Time to clear 50% parasitemia (d)			
Mean ± SD	0.72 ± 0.02	0.97 ± 0.03	< 0.0000001
Range	0.50–2.00	0.5–2.5	
95% CI	0.7–0.8	0.9–1.0	
Time to clear 90% parasitemia (d)			
Mean ± SD	1.30 ± 0.04	1.74 ± 0.06	< 0.0000001
Range	0.9–3.6	0.9–4.5	
95% CI	1.2–1.4	1.6–1.9	
Parasite clearance time (d)			
Mean ± SD	1.4 ± 0.6	2.1 ± 1.0	< 0.0001
Range	1–4	1–5	
95% CI	1.3–1.5	1.8–2.3	
Day and responses (S/RI/RII*†)			
14	171/0/0	168/3/0	0.24
21	169/2/	165/6/	0.28
28	166/5/	156/15/	0.038
35	163/8/	154/17/	0.09
42	162/9/	152/19/	0.048‡
ACPR§	162	152	
LPF	9	10	
LCF	0	9	
ETF	0	0	
PCR-corrected cure rate (%)	166 (97)	161 (94)	0.29

Cure rates on Days 14, 21, 28, 35, and 42 for AMQ-treated children were 100%, 98.8%, 97.1%, 95.3%, and 94.7%, respectively. For MQ, the corresponding values were 98.2%, 96.5%, 91.2%, 90%, and 88.8%, respectively.
* PCR-uncorrected.
† No RII or RIII response in AMQ or MQ groups.
‡ By Mantel-Haenszel test.
§ Adequate clinical and parasitologic response (on Day 42).
AMQ = artesunate-mefloquine; MQ = mefloquine; LPF = late parasitologic failure; LCF = late clinical failure; ETF = early treatment failure.

(recrudescence or re-infection) between 2 and 6 weeks after start of therapy was significantly lower with AMQ at 4 and 6 weeks ($P < 0.05$; Table 2). All of the patients were symptomatic within 10 days of reappearance of parasitemia.

PCR findings. Matched sample pairs collected before and after treatment from 27 of 28 patients in whom infections reoccurred after treatment with MQ and AMQ were successfully analyzed at MSP-1, MSP-2, and GLURP loci. Presence of different allelic families of MSP-1 and MSP-2 was often found in parasite DNA derived from a single patient, indicating a

Table 3
Gametocyte carriage in children with *P. falciparum* malaria before and after treatment with mefloquine or artesunate-mefloquine

Treatment group (no. of children)	No (%) of patients with gametocyte appearance			*P* value
	At enrollment	After enrollment	Total	
Mefloquine (171)	20 (11.4)	44 (25)	64	0.001
Artesunate-mefloquine (171)	22 (12.6)	22 (12.6)	44	0.87
Total	42 (12)	66 (18.9)	108	

polyclonal infection. Ten of 18 (56%) paired pre- and post-treatment samples obtained from patients who showed re-appearance of parasitemia after treatment with MQ showed identical allelic families of *msp-1*, *msp-2,* and *glurp*, indicating genuine treatment failures. Paired samples from the remaining eight patients (44%) showed different allelic families of *msp-1*, *msp-2,* and *glurp* and were classified as newly acquired infections after MQ treatment. Polymorphic loci of *msp-1, msp-2,* and *glurp* in paired pre- and post-treatment samples of patients who showed reoccurrence of infections after treatment with AMQ confirmed genuine recrudescent infections in five of nine patients, whereas four were classified as newly acquired infections after genotyping. Overall, the PCR-corrected cure rate was 97% for AMQ and 94% for MQ.

Adverse events. Overall, 44 children (32 boys and 12 girls), 20 in the AMQ group and 24 in the MQ group, reported at least one adverse event within the first week of starting treatment. There was no significant difference in the proportions of patients reporting adverse events in both treatment groups ($\chi^2 = 0.43$, $P = 0.51$). However, the proportion of male children reporting adverse events was significantly higher than that of female children ($\chi^2 = 8.91$, $P = 0.002$). No child reported more than two adverse events. Many of the children reporting adverse events were > 5 years of age (24 of 44). Table 4 is a summary of adverse events reported within the first week. Intravascular hemolysis (marked drop in hematocrit from 28% to 17% and hemoglobinuria) requiring hospitalization for 6 days occurred on Day 3 in a 6-year-old male child treated with AMQ. There were no other significant clinical findings in this patient. However, glucose-6-phosphate dehydrogenase (G6PD) status was not determined in the patient. Recovery was uneventful. All adverse effects were reversible, and there was no evidence of balance or hearing impairment attributable to artesunate.

Drug-attributable fall in hematocrit and resolution of anemia after treatment. Hematocrit data were available in 245 children, 119 in the AMQ group and 126 in the MQ group, at enrollment. Hematocrit values were available in 191 children, 89 in the AMQ group and 102 in the MQ group, on both Days 0 and 3, and these were used for the assessment of DAFH. In these children, there was no change in hematocrit values between Days 0 and 3 in seven and nine children, respectively, in the AMQ and MQ groups. In 15 and 10 children in the AMQ and MQ groups, respectively, there was an increase in hematocrit values between days 0 and 3. In 66 and 83 children in the AMQ and MQ groups, respectively, there was a decrease in hematocrit values between days 0 and 3. There were no significant differences in these proportions between the two treatment groups. DAFH was significantly greater in MQ- than AMQ-treated children (6.1 ± 4.5; 95% CI = 5.2–7.0 versus 4.8 ± 3.9%; 95% CI = 3.6–6.0; $P = 0.03$; Figure 2). In general, the rates of rise in hematocrit in the two treatment groups were similar after Day 3 (Figure 2).

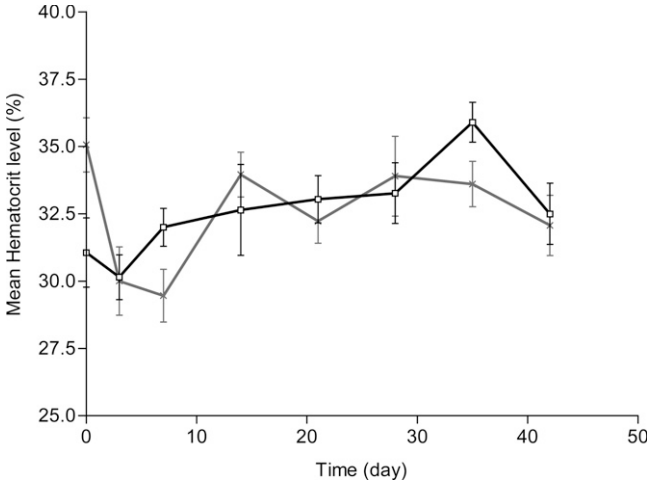

FIGURE 2. Changes in hematocrit before, during, and after treatment with artesunate-mefloquine (0) and mefloquine (×) in children with falciparum malaria.

Overall, 76 children were considered anemic (PCV < 30%) at presentation: 43 in the AMQ group and 33 in the MQ group. Ten of the 76 children with anemia were gametocyte carriers at presentation, whereas 18 of the 169 children without anemia were gametocyte carriers at presentation. The difference between the two proportions was not significant ($\chi^2 = 0.39$, $P = 0.53$). Table 5 is a summary of the rate of resolution of anemia after treatment in 66 children in whom complete data were available from Days 0 to 42. These rates were similar for both treatment groups.

Re-treatment of treatment failures. Of the 28 children with reappearance of parasitemia during follow-up, 7 each were retreated with AMQ or MQ. In all children, parasitemia cleared

TABLE 4
Adverse events reported within the first week of the study

	AMQ	MQ
No. of children	171	171
Adverse event		
Pruritus	0	2
Vomiting	8	14
Abdominal pain	6	6
Weakness	2	1
Sleeplessness	2	2
Diarrhea	1	2
Headache	0	1
Icterus	0	1
Intravascular hemolysis	1*	0
Excessive salivation	0	2

* Male child 6 years of age, G-6-PD status not determined; required hospitalization.
AMQ = artesunate-mefloquine; MQ = mefloquine.

TABLE 5
Resolution of malaria-associated anemia after treatment

	AMQ	MQ	*P* value
No. with PCV < 30%	34 ($N = 108$)	32 ($N = 112$)	0.74
Mean PCV (%) and [range]	24.9 [17–29]	25.2 [18–29]	
No of males (%)	20 (58.8.1)	16 (50)	0.50
Age (months)			1.0
Mean ± SD	61.5 ± 35.4	67.9 ± 36.5	
Range	12–120	10–132	
Number < 60 months	16	12	0.59
Parasite count (/μL)			
Geometric mean	46,183	51,399	
Range	2,800–503,200	6,900–346,875	
No. with gametocytemia at presentation (%)	3 (5.3)	7 (12.2)	0.36
No with PCV < 30(%)			
Day 7	13 ($N = 24$)	15 ($N = 19$)	0.17
Day 14	7 ($N = 16$)	4 ($N = 14$)	0.63
Day 21	3 ($N = 13$)	2 ($N = 7$)	1.0*
Day 28	5 ($N = 11$)	1 ($N = 7$)	0.31*
Day 35	2 ($N = 9$)	3 ($N = 8$)	0.61*
Day 42	3 ($N = 9$)	1 ($N = 6$)	0.6*

* Fisher exact test.
AMQ = artesunate-mefloquine; MQ = mefloquine.

within 2 days and did not recur during another 42 days of follow-up. PCR analysis showed that these were re-infections.

DISCUSSION

Both AMQ and MQ proved to be effective treatments for acute, uncomplicated *P. falciparum* malaria in this endemic area where innate resistance to MQ was reported > 20 years ago.[12,13] The insignificant decline in MQ efficacy compared with the findings 20 years ago and despite the reported innate resistance could be because of a number of reasons. First, as previously suggested,[14] there may be a lack of predictive value of performance *in vivo* by *in vitro* data. Second, there may be a play of the inverse relationship in sensitivity *in vitro* in isolates of *P. falciparum* to MQ and CQ,[14,29] because CQ resistance is currently high grade in the area.[30] Third, until recently, the very limited use of MQ in the area since the early 1990s may have allowed restoration of sensitivity to isolates with reduced sensitivity *in vitro*. Thus, the efficacy of MQ in the first 2 weeks after starting treatment, in very young children, in this study is nearly similar to that in the same area almost 20 years ago.[17] However, there was a significant decline in efficacy after 2 weeks, indicating MQ-resistant parasites are present in the area. In very young children from another endemic setting in West Africa, the efficacy of this drug has been compromised because of low blood levels after oral administration.[31]

As anticipated, the results with AMQ were better than with MQ alone despite the relative absence of a significant decline in *in vivo* sensitivity to MQ. The initial rapid responses, characterized by significantly rapid clearance of asexual parasitemia with AMQ were clearly caused by the artemisinin derivative, artesunate. The latter ensured rapid recovery and reduced the risk of reappearance of parasitemia and prevented the risk of deterioration to severe malaria that may sometimes occur with slow-acting schizonticides. The artesunate in AMQ also significantly reduced the intensity of gametocytemia, as previously reported by others,[32–34] which may contribute to prevent transmission of the infection. Although this impact may not be readily obvious in areas of intense transmission,[35] there may be significant impact on malaria incidence in areas of low transmission.[7,36] The sensitivity of *P. falciparum* to AMQ seems encouraging in the very few areas of Africa where it has been evaluated and in many areas elsewhere.[32,34,37–41] However, in certain areas, for example, along Cambodia–Thailand and northwestern border of Thailand, the efficacy of AMQ is declining primarily because of resistance to MQ.[42,43] Recently, artemisinin resistance has been reported in Cambodia.[44] Thus, close monitoring will be needed with the use of AMQ in those areas of West Africa where reduced *in vitro* susceptibility has been reported to MQ and artemisinin[13] and where patients are more likely to take the artesunate and leave the MQ component or the possibility of providers selling one drug to reduce costs in co-packaging now available in many endemic areas of West Africa. In addition, the long half-life of mefloquine poses a risk of selection of resistant parasites later in the recovery phase when dug levels are low.

Except for possibly drug-related intravascular hemolysis, both drug treatments seem well tolerated. In general, many of the reported adverse events could be attributable to MQ and were indistinguishable from those of malaria; all were reversible. There was no evidence of balance or hearing impairment attributable to artesunate. The overall rate of adverse effects was 13% and was significantly higher in older children and in boys. This rate may be an underestimate because younger children are less likely to volunteer adverse effects compared with older children. Adverse effects of MQ, for therapy or prophylaxis, are usually more frequent in girls[45–50] but may be unrelated to drug levels.[48] It was surprising that adverse effects were more common in male children and the reasons are unclear. Sex-related difference in drug metabolism has been described for a number of drugs[51] but not for MQ when administered with or without artesunate in non-pregnant women. However, such differences, if they exist, may lead to higher blood levels in the male child and may explain the higher rate of side effects. Hormonal factors may also contribute to this difference. In contrast to that seen in adults from the same area after MQ treatment,[52] adverse central nervous side effects were uncommon in the children evaluated.

Despite a significantly higher mean hematocrit value at enrollment in children treated with MQ, MQ produced a significantly greater fall in hematocrit during treatment compared with AMQ. This would suggest that the spleen may not destroy all parasitized red blood cells whose parasites have been killed by AMQ. This finding lends support to that of Chotivanich and others,[53] who showed that artemisinin derivatives may selectively kill parasites without the spleen destroying the red cells containing the dead parasites. However, it is possible that the addition of MQ to artesunate may attenuate the favorable effects of artesunate alone on red cell preservation during the removal of dead parasites as has been shown for the addition of amodiaquine to artesunate.[54]

Overall, 4% of all anemic children at enrollment were still anemic 4–6 weeks after starting therapy. Despite a significantly higher drug-associated fall in hematocrit in MQ-treated children, this rate of recovery was not influenced by artesunate—an observation consistent with that of others from another endemic area in Africa.[55] This would suggest that other factors contributing to the anemia seen in children with uncomplicated *P. falciparum* malaria need to be explored.

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