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Use of area under the curve to evaluate the effects of antimalarial drugs on malaria associated anemia after treatment

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Abstract

To evaluate the effects of antimalarial drugs on *Plasmodium falciparum* malaria associated anemia (MAA), we use the area under curve (AUC) of anemia levels after treatment as an approach to combine their duration and magnitude. The method involves numerical estimation, by trapezoidal rule, of AUC from a plot of deficit in hematocrit levels from 30% (the lower threshold of normal) *versus* time in anemic children. Using the method, we evaluated, in randomized trials, the effects of artesunate-mefloquine (AMQ) *versus* mefloquine alone (MQ), and artemether-lumefantrine (AL) *versus* amodiaquine-artesunate (AA) on the time-course of recovery from MAA in 109 children. Anemia resolution times were similar (10.9 ± 6.2 [SD] vs 13.3 ± 8.9 d, P = 0.2) but mean AUC was significantly lower in AMQ- compared to MQ- treated children (35.5 ± 7.1 [SEM] vs 49.8 ± 11.3 %.h, P = 0.02) indicating larger exposure to anemia in MQ-treated children. In AL- and AA- treated children, both anemia resolution times (8.6 ± 5.3 [SD] vs 8.6 ± 4.8 d, P = 0.98) and mean AUC (57.1 ± 12.9 [SEM] vs 46.3 ± 8.7 %.h, P = 0.74) were similar. Estimation of AUC appears more robust than estimation of anemia resolution time in evaluating antimalarial drug effects and can be used in both observational studies and clinical trials assessing the effects of therapies on MAA.

Keywords

area under curve (AUC); malarial anemia; antimalarial drugs; children

Introduction

Malaria is a significant cause of anemia in children living in areas of high and low intensities of transmission[1-4]. In these children, the prevalence of malarial anemia is over 30%[4], and in the future, it is likely the prevalence and intensities of malarial anemia will increase as drug resistance in *Plasmodium falciparum*, already reported to artemisinin in Asia[5], increases and spreads to Africa and elsewhere. The anemia in malaria has been attributed to destruction of infected and non-infected red cells and variable degree of

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dyserythropoiesis[2, 6-8] and both acute and sub-acute infections contribute to the morbidity and mortality associated with malarial anemia [9,10].

Anemia, in malaria, is evaluated by measuring hematocrit or hemoglobin values, and other hematological indices and by microscopic evaluation of red blood cell morphology. Evaluation is by serial measurement of these parameters on the day of treatment and on 3 or 7, 14, 21, 28, and if necessary, 35, and 42 days after start of treatment. Studies frequently evaluate the impacts of antimalarial drugs on malarial anemia by measuring anemia rates on these days or change in hematocrit or hemoglobin values from baseline[2, 11-13]. Typically two descriptors of anemia distribution in malaria have been used: 1) the proportion of individuals with anemia, and the degree of anemia, namely mild, moderate, or severe at a particular day after treatment, and 2) the time taken from start of treatment until anemia resolves (anemia resolution time).

We propose the use of an alternative outcome for malaria-associated anemia (MAA) that can incorporate magnitude and duration of anemia in anemic children enrolled in observational studies and clinical trials: the area under curve of anemia levels versus time after treatment with antimalarial drugs. The main advantage of the approach is that the outcome incorporates longitudinal data of hematocrit or hemoglobin levels collected at several days.

We examined this analytical approach using data from two prospective studies designed to evaluate the resolution of MAA in children treated with artemisinin-based combination therapies (ACTs) and a non ACT in a region where MAA is common.

Materials and Methods

Study area

The study was carried out in Ibadan, southwest Nigeria from July 2007 to September 2009. In this area of hyperendemic malaria, transmission occurs all year round but is more intense during the rainy season from April to October[14]. Estimates of MAA in the area vary between 20-70%[12, 15].

Study population

The study patients were enrolled at the malaria clinic of the University College Hospital, Ibadan, Nigeria. Patients were included if their attending relatives gave informed written consent, they were aged 0.5 to 15 years, had single species asexual *P. falciparum* parasitemia ≥ 2000/μL, did not have a significant history of antimalarial drug intake in the 2 weeks preceding presentation, and had a good likelihood of being able to complete 6 weeks of follow-up. Patients with severe malaria[16], severe malnutrition, serious underlying diseases (renal, cardiac, or hepatic), and known allergy to study drugs were excluded from the study. The study protocol was approved by the Ethics Committee of the Ministry of Health, Ibadan, Nigeria.

Drug Management

After clinical assessment, blood was obtained for hematocrit determination and for quantification of asexual and sexual parasitemia. Patients were randomized to receive standard doses of antimalarial drugs (Table 1). All drugs were given orally and all patients waited for at least 3 h after to ensure the drug was not vomited. If it was, the patient was excluded from the study. Oral acetaminophen at 10-15 mg/kg 6 hourly was given for 12-24 h if body temperature was > 38°C. Patients were seen daily, at approximately the same time of the day for the first eight days (days 0-7) and then daily on days 14, 21, 28, 35 and 42

after treatment had begun. At each visit, patients were assessed clinically and thick and thin blood smears were obtained for quantification of parasitemia.

The fever clearance time was defined as the time taken for the body temperature to fall to below 37.5°C and remain below this value for > 48 h.

Laboratory investigations

Asexual parasite and gametocyte counts were measured daily for the first eight days (days 0-7) and thereafter on days 14, 21, 28, 35 and 42. Quantification in Giemsa-stained thick blood films was done against 500 leukocytes in the case of asexual parasitaemia, and against 1000 leukocytes in the case of gametocytes, and from these figures, the parasite density was calculated assuming a leukocyte count of 6000/μl of blood. Parasite clearance time was the time interval from the start of antimalarial treatment until the asexual parasite count fell below the detectable levels in a peripheral blood smear. Capillary blood collected before and during follow-up, was used to measure packed cell volume or hematocrit. Hematocrit was measured using a microhematocrit tube and microcentrifuge (Hawksley, Lancing, UK). Hematocrit was done on days 0-7, 14, 21, 28, 35 and 42.

Anemia was defined as a hematocrit value < 30% and in anemic patients, anemia resolution time as the time elapsing from treatment to the attainment of a hematocrit ≥ 30%.

Outcome variable: AUC of anemia after treatment

The area under curve (AUC) is frequently used in clinical pharmacology to estimate the area inscribed by the plot of plasma, serum or whole blood drug levels *versus* time and can be interpreted as the total uptake or extent of exposure to drug. It is a summary calculation used when serial measurements on each subject under study are carried out.

In all anemic patients at enrolment, hematocrit values below 30% (the lower threshold of normal) and at follow up-were subtracted from 30% at each time of measurement until hematocrit rose to 30%, and the resulting values plotted against time. The final hematocrit when anemia resolved was therefore zero in all patients. The areas under the curve (AUC) of deficit in hematocrit (from 30%) *versus* time were obtained, by the trapezoidal rule using the computer program *Turbo Ken* (designed by Clinical Pharmacology Group, University of Southampton, United Kingdom). If there was no resolution of anemia during follow-up, AUC was calculated until 1008 h (day 42). AUC was also be obtained manually by calculating the average hematocrit values between two consecutive time measurements and multiplying it by the time interval between the measurements, and summing up all the values, in a manner similar to that for the numerical estimation of area under a drug concentration-time curve[17]. Both measurements by digital computer and manual methods gave the same values. The unit of quantification would be %.h, if hematocrit values were used or g.h/dL if hemoglobin values were used. Hematocrit values may be converted to hemoglobin values by dividing by 3.

Data analysis

Data were analyzed using version 6 of the Epi-Info software[18], and the statistical programme SPSS for Windows version 10.01[19]. 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 by Mantel Haenszel tests. Normally distributed, continuous data were compared by Student's 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 were two-tailed. P-values of ≤ 0.05 were 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

Demographic and clinical characteristics at enrolment

Between July 2007 and September 2009, 652 children were randomized to receive MQ or AMQ (n = 342), or AL or AA (n = 310). There were 350 girls. Anemia was present in 109 of 435 children in whom hematocrit values were available at enrolment and follow-up. It was mild or moderate (haematocrit values of 21-29% or 15-20%, respectively), in 99 or 10 children, respectively. No child had severe anemia (hematocrit < 15%).

Clinical outcomes

All children recovered, clinically, from their illness. Fever and parasitemia cleared in all children, irrespective of treatment within 2-5 days. The details of the treatment outcomes for AMQ and MQ have been reported elsewhere[13]. The details of the treatment outcomes for patients treated with AL and AA will be reported elsewhere. The outcomes were similar in children treated with AA co-formulated and AA co-packaged. Therefore the data from children treated with AA were combined.

Comparison of AUC in children treated with MQ and AMQ

The characteristics of the children with anemia who were treated with MQ or AMQ are shown in Table 2. These characteristics, the proportions of individuals with anemia during follow-up, and the anemia resolution times (13.3 ± 8.9 [SD], range 3-35 vs 10.9 ± 6.1 d, range 3-14, P = 0.2) were similar in the two treatment groups.

Figure 1 shows the mean AUC and the distribution of individual AUC in the two treatment groups. Mean AUC was significantly higher in those treated with MQ compared with those treated with AMQ (49.8 ± 11.3 [SEM], range 0.43-165.4 vs 35.5 ± 7.1, range 0.41-161.9 %.h, P = 0.02). The proportion of children with AUC > 30 %.h was also significantly higher in children treated with MQ than in those treated with AMQ (17 of 32 vs 8 of 34, $\chi^2 = 4.94$, P = 0.02).

Comparison of AUC in children treated with AL and AA

The characteristics of the children with anemia who were treated with AL or AA are shown in Table 3. These characteristics, the proportions of individuals with anemia during follow-up, and the anemia resolution times (8.6 ± 5.3 [SD], range 3-21 vs 8.6 ± 4.8 d, range 3-21, P = 0.98) were similar in the two treatment groups.

Figure 2 shows the mean AUC and the distribution of individual AUC in the two treatment groups. Mean AUC (57.1 ± 12.9 vs 46.3 ± 8.7 [SEM] %.h, P = 0.74) and the proportion of children with AUC > 30%.h were similar in the two treatment groups of (11 of 18 vs 12 of 25, $\chi^2 = 0.29$, P = 0.58).

Discussion

Accelerated clearance of asexual forms of *P. falciparum* and resolution of symptoms and signs of infection are the major goals of antimalarial therapy at the individual patient level. However, since anemia is an inevitable consequence of untreated, (and in many cases of treated drug-resistant) falciparum malaria, a risk factor for gametocyte carriage[20-22] and indirectly transmission, and because antimalarial drugs may influence the time-course of anemia[2, 12], it has become increasingly recognized that anemia is a public health outcome

measure of the disease and of antimalarial drug treatment. Traditionally, this outcome has been evaluated in cross sectional analyses that compare hemoglobin distributions and anemia prevalence in different age and population groups[23]. Duration of anemia may also be evaluated with a cohort approach as the interval between the first and the last detection or appearance of anemia, or by the time of first detection of anemia in those who were not previously anemic at the start of treatment. A possible but not used approach during follow-up is to estimate anemia person week by adding presence of anemia among individuals under follow-up to estimate anemia person week in a manner similar to that used for estimating gametocyte person week[24].

The main advantage of the method we present is that it succinctly incorporates both duration and magnitude of anemia into a single measure defined by AUC. Furthermore, by subtracting the measured hematocrit from that defined by the lower threshold of normal value, a quantitative estimate of the burden of anemia was produced. AUC also permitted analysis in two stages: first, the characterization of the presence of anemia and second, the magnitude of anemia among those in whom anemia was present.

There are other advantages over the conventional methods in using AUC to determine the effects of antimalarial drugs on MAA after treatment of the individual: point prevalence of anemia and point measurement of hematocrit do not represent the most accurate estimates of anemia load over time; the effects exerted by parasite load, drug and other factors may vary significantly over time; in drug studies, point estimates of resolution rates or of anemia resolution times, while of much value may not be sensitive enough to detect subtle differences in drug effects in controlled trials.

Anemia resolution times in those treated with AMQ and MQ were similar. Indeed, anemia resolution times were similar for all treatment regimens evaluated in our cohort of children. These findings are similar to those of a previous study in the same endemic area that compared anemia resolution times in children treated with artesunate, amodiaquine or artesunate-amodiaquine[12]. However, the mean value of AUC was significantly higher and one-and a half fold greater in MQ-than in AMQ-treated children. In addition the proportion of children with AUC > 30 %.h in MQ-treated children was significantly higher than in AMQ-treated children. Taken together, these would suggest 1) significantly longer exposure to anemia in MQ-treated children, and 2) anemia resolution time may be less sensitive than AUC in eliciting subtle differences in the effects of antimalarial drugs on MAA in these children. Additionally, the results suggest that ACTs may significantly reduce AUC compared with a non-ACT. Thus, estimates of AUC may be a more robust method for comparative assessment of the effects of antimalarial drugs on MAA than the conventional anemia resolution time.

The favorable effects of ACTs on MAA may have been due mainly to their ability to rapidly increase the number of ring infected surface antigen (RESA) positive, parasite negative red cells in circulation compared to non ACTs[25], although the life span of these cells (approximately 83 d) may be significantly shorter than the general pool of red cells[8]. This would translate to a lesser fall in hematocrit within the first few days of commencing treatment[12, 13], and to a lesser than expected fall in hematocrit in patients with hyperparasitemia.

The pathogenesis of MAA is complex and incompletely understood[2, 6, 7, 26]. The processes of recovery from MAA are influenced, among others, by the rapidity and stage specificity of antimalarial drug action, the life span of the different pools of erythrocytes present in the infected patients[8, 25], and the proclivity or otherwise of the antimalarial drugs to induce hemolysis in the patient. In the last context, the partner drug in ACT may influence their

ability to reduce quickly the degree and severity of MAA. For example, Premji et al.[27] have shown that despite the non-inferiority in antimalarial efficacy of chlorproguanil-dapsone–artesunate combination to AL in children treated with these ACTs, the significant hemolysis and subsequent anemia caused by the dapsone component of chlorproguanil-dapsone-artesunate in glucose-6-phosphate dehydrogenase-deficient children does not make chlorproguanil-dapsone-artesunate suitable for use in public health setting in Africa.

The application of AUC following antimalarial treatment need not be limited to use of hematoctrit values. AUC may also be estimated using hemoglobin concentrations measured over time, but hemoglobin estimation requires more expensive items of equipment and greater expertise than are required for hematocrit estimation. Application may also be extended to individual hematological parameters such as mean corpuscular hemoglobin concentration. Thus, the application of the principle for the effects of drugs on MAA is potentially wide.

In conclusion, we have demonstrated in this study how AUC can be used to evaluate the effects of antimalarial drugs on MAA. The method is applicable to both observational studies and clinical trials assessing the effects of therapies on MAA. Thus, it may be useful for clinical trials that compare the efficacy of antimalarial drugs used alone or in combination to incorporate the estimation of AUC in order to define which treatments would potentially be better able to lessen the burden of MAA.

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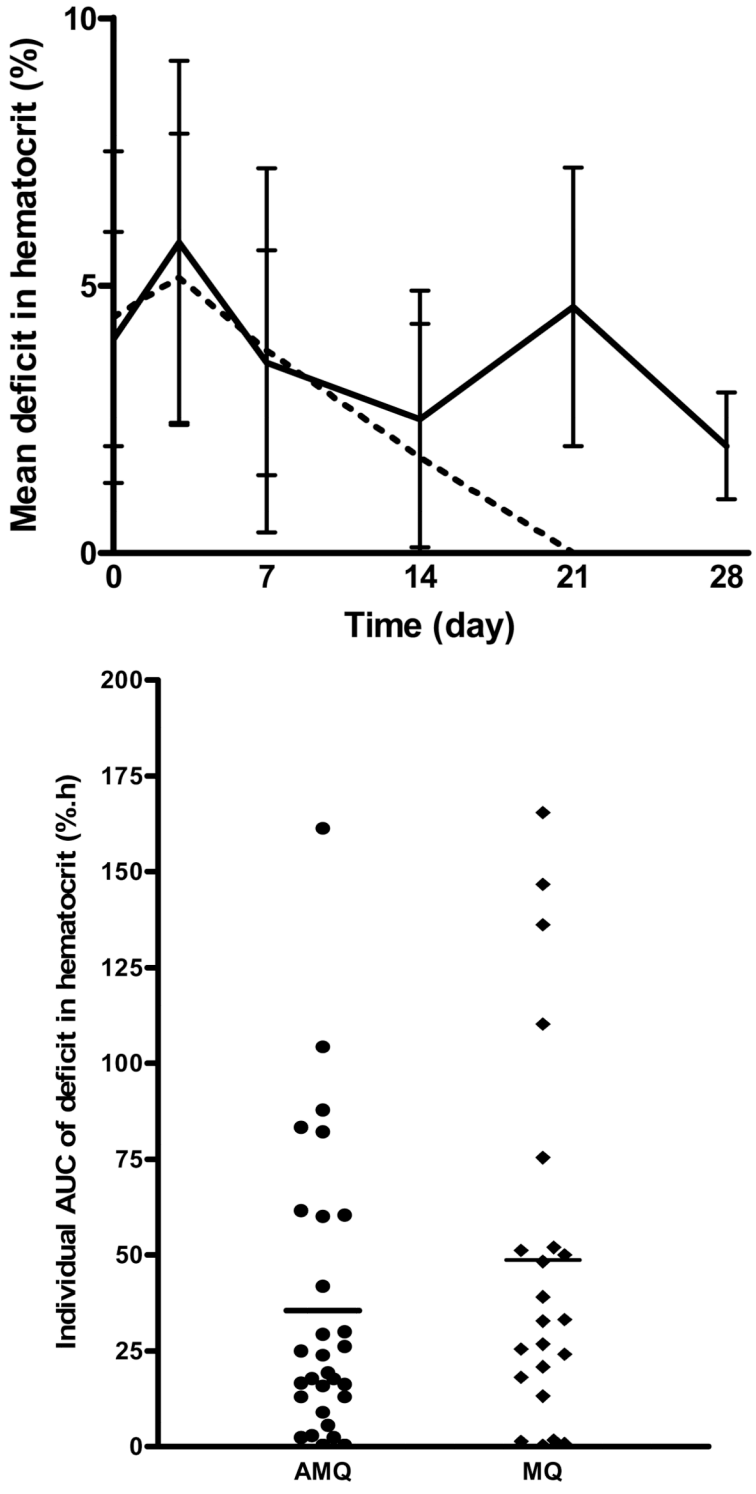

Figure 1.

Figure 1 a. Area under curve of mean deficit in hematocrit in patients treated with artesunate mefloquine (---) or mefloquine alone (-)

Figure 1 b. Distribution of individual AUC in patients treated with artesunate-mefloquine (AMQ) [●] or mefloquine alone(MQ) [▲].

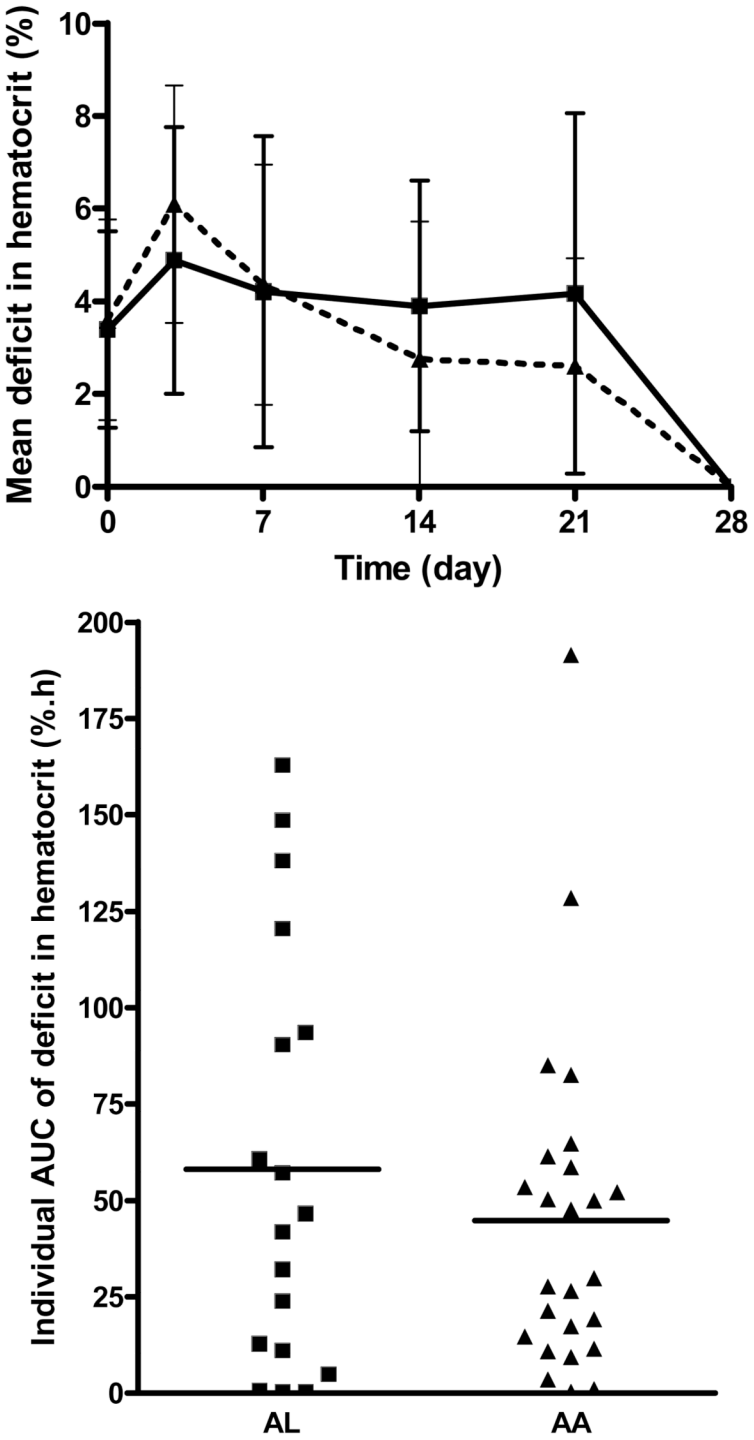

Figure 2.

Figure 2 a. Area under curve of mean deficit in hematocrit in patients treated with artemether-lumefantrine (-■-) or artesunate-amodiaquine (-▲-)

Fig 2 b. Distribution of individual AUC in patients treated with artemether-lumefantrine (AL) [■] or artesunate-amodiaquine (AA) [▲].

Table 1

Treatment regimens and time of study in the children enrolled

Drugs*	Regimens†	No of patients	Year
AMQ	Mefloquine 25mg/kg at presentation plus artesunate 4mg/kg daily for 3 days	171	2007-2008
MQ	Mefloquine 25mg/kg at presentation	171	2007-2008
AL	Artemether (20mg) plus lumefantrine (120mg) given according to body weight: 5-14kg received 1 tab., 15-24kg received 2 tab., 25-34kg received 3 tab., > 34kg received 4 tab. at presentation, 8 h later and at 24, 36, 48 and 60 h after first dose	129	2009
AAcp**	Artesunate 4 mg/kg daily for 3 d plus amodiaquine 10mg/kg daily for 3 d (co-packaged) or according to age: 1-5 years received 1tablet each of AA,; 6-10 years receieved 2 tablets each; 11-15 years received 3 tablets each.	84	2009
AAcf***	Co-formulated amodiaquine plus artesunate given as follows: children weighing ≥ 9 - < 18 kg or aged 1-5 years received 0.5 tablet; children weighing ≥ 18 - 36 kg or aged 6-13 years received 1 tablets; children weighing ≥ 36 kg or aged ≥ 14 years received 3 tablets	85	2009

† All drugs were administered orally. AAcp Amodiaquine plus artesunate co-packaged; AAcf Amodiaquine plus artesunate co-formulated; AL artemether-lumefantrine; AMQ artesunate plus mefloquine; MQ, mefloquine;

** Each tablet of amodiaquine contains 153 mg base and each tablet of artesunate contains 50 mg.

*** Each co-formulated tablet contains artesunate 100 mg and amodiaquine base 270 mg.

Table 2

Resolution of malaria-associated anemia following treatment with artesunate-mefloquine or mefloquine

	AMQ	MQ	P. value
No. with HCT < 30%	34 (n = 108)	32 (n =112)	0.74
Mean HCT (%) and [range]	24.9 [17 – 29]	25.2 [18 – 29]	
No of males (%)	20 (58.8.1)	16 (50)	0.50
Age (months)			
mean ± sd	61.5 ± 35.4	67.9 ± 36.5	1.0
range	12– 120	10 – 132	
No. < 60 months	16	12	0.59
Parasite count (/μL)			
geometric mean	46183	51399	
range	2800 – 503200	6900 – 346875	
No. with gametocytaemia at presentation (%)	3 (5.3)	7 (12.2)	0.36
No with HCT < 30 (%) on			
Day 7	13 (n = 24)	15 (n = 19)	0.17
14	7 (n = 16)	4 (n = 14)	0.63
21	3 (n = 13)	2 (n = 7)	1.0*
28	5 (n = 11)	1 (n = 7)	0.31*
35	2(n = 9)	3 (n = 8)	0.61*
42	3 (n = 9)	1 (n = 6)	0.6*
Anemia resolution time (d)	10.9 ± 6.2	13.3 ± 8.9	0.2
Time to 50% resolution	5.4 ± 3.0	6.6 ± 4.4	0.2
Time to 90% resolution	9.8 ± 5.5	11.9 ± 7.9	0.2

HCT, hematocrit; AMQ, artesunate-mefloquine; MQ, mefloquine

*Fisher exact test

Table 3

Resolution of malaria-associated anemia following treatment with artemether-lumefantrine or artesunate-amodiaquine

Variable	AL	AA	P value
No. with HCT < 30%	18 (n = 129)	25 (n = 169)	0.96
mean (range)	26.6[17 – 29]	26.4[20 – 29]	
No. of males (%)	7[38.9]	12[48]	0.78
Age (yr)			
Mean ± sd	5.94 ± 3.6	5.3 ± 2.5	0.51
Range	1 – 13	2 – 10	
Parasite count(/μl)			
Geometric mean	53,712	75,853	0.54
Range	2,057-240,780	12,654-288,461	
No. with gametocytes at presentation (%)	0 (0)	4 (16)	0.12*
No. with HCT < 30% on			
D7	7(n = 12)	11(n = 21)	0.97
D14	4(n = 9)	3(n = 21)	0.15*
D21	3(n = 10)	1(n = 17)	0.13*
D28	2(n = 7)	0(n = 5)	0.47*
D35	1(n = 5)	0(n = 4)	1.0*
D42	1(n = 6)	0(n = 3)	1.0*
Anemia resolution time (d)	8.6 ± 5.3	8.6 ± 4.8	0.98
Time to 50% resolution (d)	4.3 ± 0.63	4.3 ± 2.4	0.98
Time to 90% resolution (d)	7.7 ± 4.8	7.7 ± 4.3	0.98

HCT, hematocrit; AL-artemether-lumefantrine; AA-artesunate-amodiaquine;

*Fischer exact test.