A Randomized Trial of Artesunate Mefloquine versus Artemether Lumefantrine for the Treatment of Uncomplicated *Plasmodium falciparum* Malaria in Senegalese Children

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Abstract. An open randomized clinical trial study was carried out to compare efficacy and tolerability of artesunate mefloquine 25 mg/kg body weight (Artequin paediatric) versus artemether lumefantrine (Coartem) in the treatment of uncomplicated *Plasmodium falciparum* malaria in children. In each arm, 160 patients were assigned to receive either AS + MQ or AL with 28 days follow-up. The adequate clinical and parasitological response at Day 28 for per protocol analysis was after polymerase chain reaction correction, 100% for AS + MQ and 96.8% for AL. In the intention-to-treat analysis, the respective cure rates were 96.2% for AS + MQ and 93.7% for AL. No serious adverse events (AEs) were reported. The most frequent AE was vomiting, 30% in AS + MQ arm and 36% in AL arm. No biological significant abnormal values related to the study drug have been reported. The new pediatric artesunate mefloquine formulated in granule fixed dose combination is well adapted to children in Africa.

INTRODUCTION

Because the World Health Organization (WHO) has recommended artemisinin combination therapies (ACTs) for the treatment of malaria, several ACTs have been evaluated and are now widely used.¹ However, only a few pediatric formulations are available for young children who are suffering more from malaria. Artesunate-mefloquine is an ACT that has been evaluated in many studies with a dosage of mefloquine at 15 mg/kg body weight (bw). In west Africa this combination is widely used, its efficacy and safety have been demonstrated so far.^{2,3} The WHO recommends to use mefloquine at 25 mg/kg to avoid appearance of resistance. Data are limited with this dosage in Africa. To address the issues of dosage and pediatric formulations, a new artesunate + mefloquine formulation was developed by MEPHA (Artequin paediatric). It is a preparation of granules of 50 mg of artesunate and 125 mg of mefloquine as a fixed-dose combination and it is suitable for children with body weight ranging from 10 to 20 kg. This new formulation at 25 mg/kg of mefloquine is currently used in many African countries. Although, most of the one arm treatment designed studies conducted with this new formulation have shown high efficacy and safety.³ No randomized trials using this new dosage have been conducted so far. We carried out this study with the objective to compare the efficacy and tolerability of the new Pediatric artesunate mefloquine combination versus artemether lumefantrine (Coartem, Novartis, Pharma Ltd., Basel, Switzerland) in the treatment of uncomplicated *Plasmodium falciparum* malaria in children with body weight ranging from 10 to 20 kg.

METHODS

Study site. The study was carried out from September to December 2008 in the health centers of Ndoffane and Kaolack located from 200 km to Dakar. Malaria in this area is highly seasonal with a transmission peak from September to December and contributes to 35% of the general morbidity. *Plasmodium falciparum* is the main specie (95%). Chloroquinoreistance

is above 45% and the reported sulfadoxine-pyrimethamine and amodiaquine resistance, respectively, is 6% and 5%.⁴ The current drug policy recommends the use of an artesunate amodiaquine combination.

Study populations. We enrolled children in the study with a body weight from 10 to 20 kg who came to the local health center, with a *P. falciparum* parasite density between 1,000 and 100,000/ μ L, and presence of an axillary temperature superior to 37.5°C. Patients were excluded if they had signs of severe malaria⁵ such as convulsions, severe anemia, respiratory distress, jaundice, haemoglobinuria, or severe malnutrition⁶ (children whose weight for height was less than 70% of the median National Center for Health Statistics (NCHS)/WHO reference value, with a symmetrical oedema involving the feet), and had an allergy to study drugs or had used any component of the study drugs within 28 days of enrollment. Each participant's guardian gave fully informed written consent before enrollment. The protocol was reviewed and approved by the Conseil National de Recherche en Santé (CNRS), which is the national ethics committee.

Study procedures. It was a prospective open, randomized clinical trial with 28 days follow-up comparing 3-day regimens of AS + MQ versus AL used as a reference comparator. The WHO 2003 protocol was used.⁷ Enrolled patients were weighed and randomly assigned to receive either AS + MQ or AL. The number of patients to enroll was done through Epi info 2000 software. The expected cure rate for artemether-lumefantrine was estimated at 98%,⁸ assuming a non-inferiority of 5% and 80% power, using a 95% confidence level, sample size for each arm was 150 patients. The AL was packaged in fixed-dose combination tablets, each containing 20 mg of artemether and 120 mg of lumefantrine. The AS + MQ treatment was supplied as a three fixed pack containing 50 mg artesunate and 125 mg of mefloquine. All drug doses were administered in the health center by a physician. The AL tablets were crushed and mixed in water. The pellet of pediatric AS + MQ was directly applied into the mouth of the child. No water was needed. A full dose was re-administered if a participant vomited the study drugs within 30 minutes of initial administration. The pediatric AS + MQ was given one stickpack/sachet per day and AL was given one tablet two times per day for children with 10–14 kg bw or two tablets two times per day for children with 15–20 kg bw. The two drug regimens covered 3 days.

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TABLE 1
Primer sequence and polymerase chain reaction (PCR) program for MSP1 and MSP2 amplification

Gene	Name	Primer sequences	PCR program
Msp1 primary	O1	5' CACATGAAAGTTATCAAGAACTTGTC 3'	94°C-3 min, [94°C-25 sec, 50°C-45 sec, 68°C-2 min] × 30, 72°C-3 min
	O2	5' GTACGTCTAATTCATTTGCACG 3'	
Msp1 nested	N1	5' GCAGTATTGACAGGTTATGG 3'	94°C-3 min, [94°C-30 sec, 50°C-45 sec, 68°C-2 min] × 30, 72°C-3 min
	N2	5' GATTGAAAGGTATTTGAC 3'	
Msp2 primary	S3	5' GAAGGTAATTAACATTGTC 3'	94°C-3 min, [94°C-30 sec, 42°C-60 sec, 65°C-2 min] × 30, 72°C-3 min
	S2	5' GAGGGATGTTGCTGCTCCACAG 3'	
Msp2 nested	S1	5' GAGTATAAGGAGAAGTATG 3'	94°C-3 min, [94°C-30 sec, 50°C-60 sec, 72°C-2 min] × 45, 72°C-3 min
	S4	5' CTAGAACCATGCATATGTCC 3'	

Study participants were examined in the study clinic 1, 2, 3, 7, 14, 21, and 28 days after enrollment or at any time if they did not feel well, and thick films were done during these different visits. Quinine treatment was given in case of treatment failure.

Biological follow-up. Finger punctures to obtain blood for thick smear were done at Days 1, 2, 3, 7, 14, 21, and Day 28. Blood smears were stained with Giemsa and 200 leukocytes were read. Assuming a total leukocyte count of 8,000/ μ L, parasite density was determined as the number of asexual parasites $\times$ 8,000/200. The slide readers were kept blinded to the treatment allocated.

Polymerase chain reaction procedures. Four drops of blood were collected on filter paper at Day 0 and at day of recurrent parasitaemia to distinguish recrudescence from new infection. For this, parasite genotyping by nested polymerase chain reaction (PCR) were conducted to compare two polymorphic genetic markers from *P. falciparum*: MSP1 and MSP2 genes.

After DNA extraction, the PCR was performed using primer pairs described in Table 1. Two microliters of DNA in a total of 50 μ L of reaction mixture were amplified during the primary PCR using the program shown in Table 1. One microliter of the product of the first amplification in a total of 50 μ L of reaction mixture was used for the second PCR with the second program. Each reaction mixture contained 10 μ L of

buffer/MgCl₂ (3.5 mM), 5 μ L of dNTP (200 mM), 0.5 μ L of each primer (100 μ M), 0.25 μ L of *Taq* polymerase (0.5 units), and 32.25 μ L of distilled DNA-free water. The DNA was added last. Amplification products were visualized by photography under ultraviolet light after electrophoresis on 1.5% agarose containing Ethidium bromide for MSP 1 and 1.8% agarose for MSP2.

The difference in fragment size between pretreatment and the post-treatment samples allows a classification in three categories: new infection if the fragment sizes are different, recrudescence if the fragment sizes are similar, and both recrudescence and new infection if two fragments are visualized in the post-treatment sample with one similar to the pretreatment fragment. The last two are considered as recrudescence for the analysis.

Tolerability and safety assessment. All adverse events defined as events occurring new or worsening from baseline after administration of treatment were closely monitored. During each scheduled visit, parents or guardians were interviewed by the investigators to assess adverse events and patients were also examined by a physician. All signs noted during the interview and clinical examinations were reported on a case reported form (CRF).

Hematology and biochemical tests were done at the enrollment and at Day 7 to evaluate hemoglobin, creatinin, aspar-

FIGURE 1. Trial profile.

TABLE 2
Patients characteristics at enrollment

Characteristics	AS + MQ*	AL*	P value
Mean age (years)	4.6 ± 1.7	5.6 ± 1.6	0.25
Sex ratio (m/f)	1.6	1.6	1
Mean weight	15 ± 3.2	17.1 ± 2.7	< 10 ⁻³
Mean axillary temperature	38.2 ± 1	38.1 ± 1	0.21
Mean parasitic density	31677	37370	0.10

*AS + MQ = artesunate; MQ = mefloquine; AL = artemether-lumefantrine.

tate amino transferase (ASAT), alanine aminotransferase (ALAT), and bilirubin parameters. Biological abnormalities were noted.

Data analysis. Data were computer entered using Epi Info 2000 software. The intention-to-treat (ITT) analysis included all randomized participants who took at least one full dose and had one post-baseline efficacy assessment without major protocol violations, such as wrong weight, wrong dosage, wrong use of non-assigned drug by mistake, and co-infection with other malaria species. Patients with major violations and patients lost during the follow-up or withdrawn (caused by an adverse event or to the use of another drug with anti-malarial activity or withdrawal consent) have been considered as failure. The per protocol analysis included patients who receive the 3 doses and who have no major protocol violation up to Day 28. Those lost to follow-up and the withdrawals consent were excluded from the per protocol analysis. For the primary endpoint as the adequate clinical and parasitological response (ACPR), data were analyzed by estimations of difference in proportions corresponding to the 95% confidence interval. In addition, the cumulative risk of failure was assessed by survival analysis with the Kaplan Meier method. For the Kaplan Meier analysis, lost to follow up, withdrawals consent, and patients with re-infection were censored on the last day of follow-up. Comparison between groups was made using the χ^2 test or Fisher exact test for qualitative outcome and student test for quantitative outcome when applicable. Otherwise, non-parametric tests (Man Withney, Kruskal Wallis) were used. A P value (two-sided) less than 0.05 was considered statistically significant.

RESULTS

Disposition of study population. Of 550 screened patients, 320 met the entry criteria, 160 in each arm were assigned to

receive either AS + MQ or AL (Figure 1). Withdrawal of consent was noted in 3 patients in the AS + MQ arm and in 2 patients in the AL arm. Three patients were lost to follow-up, both in the 2 arms, caused by movement away from the site and could not be traced. Endpoint was reached in 320 patients for the intention to treat analysis and 309 patients for the per protocol analysis. (Figure 1).

Demographic and other baseline characteristics of study patients. Sociodemographic characteristics were comparable in the two groups (Table 2). Mean age of the patients was 4.6 ± 1.7 years in the AS + MQ arm and 5.6 ± 1.6 years in AL ($P = 0.25$). Sex ratio was identical in the 2 arms, 1.6 for males. Mean weight was superior in the AL arm 17.1 ± 2.7 kg versus 15 ± 3.2 kg for the AS + MQ arm. No significant difference was noted in the mean temperature.

Biological characteristics of patients at enrollment. No significant difference was noted in the mean level of hemoglobin, bilirubin, and creatinin in the two groups. The level of aminotransferase enzymes was higher in the AS + MQ arm but none significantly (Table 5).

Therapeutic Efficacy. As shown in Table 3, the ACPR at Day 28 for the per protocol (PP) analysis was 100% for AS + MQ and 96.8% for AL ($P = 0.07$). In ITT analysis, the respective cure rates were 96.2% for AS + MQ and 93.7% for AL all after PCR corrected ($P = 0.31$). Five patients were classified as recrudescence with late treatment failures after PCR in the AL arm. The Kaplan Meier survival analysis didn't show any difference between the two groups after 28 days of follow-up ($P = 0.64$) (Figure 2).

Parasite and temperature clearance profile. The two treatments resulted in a rapid clearance of parasites and fever. The mean parasitemia at enrollement was 31,677 trophozoite/μL in the AS + MQ group and 37,370 in the AL group. It decreased to 2,528 trophozoite/μL in the AS + MQ group versus 1,917 trophozoite/μL in the AL group 24 hours after the first dose ($P = 0.27$). Complete parasite clearance was obtained at Day 2 in 97.5% and 98.1% patients, respectively, for AS + MQ and AL groups ($P = 0.35$). Complete clearance of fever within 24 hours after the first dose was observed in 79% and 80% of patients in the AS + MQ arm and AL arm, respectively. At 48 hours, the complete clearance of fever was 99% in the two arms. From Day 3, no patient with fever was found for the two treatments.

Gametocyte carriage. Proportion of patients with gametocyte at enrollment was 5.8% in the AS + MQ group versus

TABLE 3
Treatment outcome of artemeter-lumefantrine and artesunate-mefloquine at Day 28

Outcome	Artemeter-lumefantrine (AL*)	Artesunate-mefloquine (AS + MQ*)	P value
Intention to treat analysis			
Enrolled patients	160	160	
Patients seen at Day 28	155/160 (96.8%)	154/160 (96.2%)	0.73
Missing	5/160 (3.1%)	6/160 (3.7%)	0.75
Crude parasitological failure at Day 28	5/160 (3.1%)	0	—
PCR adjusted failure rate at Day 28	10/160 (6.2%)	6/160 (3.7%)	0.30
PCR adjusted cure rate at Day 28	150/160 (93.7%)	154/160 (96.2%)	0.31
Per protocol analysis			
	AL	AS + MQ	
Patients seen at Day 28	155	154	
Crude parasitological failure Day 28	5/155 (3.2%)	0	—
PCR adjusted failure rate Day 28	5/155 (3.2%)	0	—
PCR adjusted failure rate Day 28	150/155 (96.8%)	154/154 (100%)	0.07

*AS + MQ = artesunate; MQ = mefloquine; AL = artemether-lumefantrine.

FIGURE 2. Kaplan-Meier estimates of efficacy in two group treatments at Day 28 (intent-to-treat [ITT] analysis). AS + MQ = artesunate-mefloquine; AL = arteleter-lumefantrine; ACPR = adequate clinical parasitological response.

3.9% in the AL group and it decreased to 2.5% and 1%, respectively, by Day 7. At Day 14, no gametocyte carriers were found (Figure 3).

Tolerability and safety. The two treatments were well tolerated during this study. No serious adverse events were reported. No patients died during the study and no signs of neurotoxicity were observed. The most frequent adverse event was vomiting 30% in the AS + MQ arm and 36% in the AL arm. The most common AEs were related to malaria. The adverse events were similar in both groups (Table 4) except for weakness and headache, which was higher in AL arm ($P < 0.001$).

Biological parameters in the two groups at D7. The mean haemoglobin level was lower at Day 7 in the 2 groups, but not significantly. Anemia was more frequent in the AL group at Day 7: 73.7% versus 65.6% in the AS + MQ group ($P < 0.001$). We noted a significant decrease and a return to normal of liver enzymes in both arms at Day 7 with no differences between treatments. A significant decrease of bilirubine level was noted in both groups. No damage was noted in the renal function as assessed by the serum creatinine measurements (Table 5).

DISCUSSION AND CONCLUSIONS

This study showed that AS + MQ was efficacious and well tolerated as AL. Results from ITT analysis indicate that the Day 28 cure rate was 96.2% in the AS + MQ arm versus 93.7%

FIGURE 3. Gametocyte carriage. AS + MF = artesunate-mefloquine; AL = artemeter-lumefantrine.

TABLE 4
Adverse events reported from Day 0 to Day 28

Adverse events	AS + MQ	AL	<i>P</i>
	(<i>N</i> = 160)	(<i>N</i> = 160)	
Weakness	135 (84%)	153 (95%)	0.001
Headache	60 (37.7%)	118 (74.3%)	0.001
Nausea-vomiting	48 (30%)	59 (36%)	0.25
Abdominal pains	7 (4.3%)	2 (1.25%)	0.09
Dizziness	5 (3.1%)	9 (5.6%)	0.36

in the AL arm after adjustment by PCR ($P = 0.31$). From per protocol analysis, the PCR corrected Day 28 cure rates were 100% for AS + MQ and 96.8% for AL ($P = 0.07$). This high cure rate confirms the efficacy of AS + MQ, in which the use is lower than other drugs in Africa. Sagara and others⁹ noted the same efficacy results in Mali, AS + MQ used with a dosage of 15 mg/kg.

The two treatments resulted in a rapid clearance of parasites and fever. All patients were free of parasites within the 48 hours. In addition, fever disappeared completely within the 48 hours. This was shown in several studies related to ACT.^{4,10,11}

The adverse events were similar in the two groups with more fatigue and headache in the AL group ($P = 0.001$). Mefloquine is associated in general with vomiting, nausea, dizziness, and insomnia. In Thailand, a comparative study showed the same levels of adverse events with the two drugs.^{11,12} It has been related to more vomiting in African children with mefloquine at 25 mg/kg. These signs were minor in our study. Vomiting is reduced when the dose is split and given in 3 days as 8.3 mg/kg/day.^{3,10,11} No neurological events were noted and no abnormal laboratory events were noted: ALAT and ASAT concentrations decreased at Day 7 and confirmed the absence of hepatic toxicity of the two treatments. No alteration of renal function was noted.

In our study, the 25 mg/kg bw dosage is efficacious and well tolerated. However, others studies must be done with higher sample size to confirm this dosage. The AS + MQ pediatric formulation in granule fixed dose combination is well adapted to young children and improves the compliance. This new dosage will avoid the appearance of resistance as was the fear with the 15 mg/kg. Another advantage of this new formulation is the administration schedule in one dose per day compared with the most pediatric ACTs, which are administered in two daily doses per day. The new formulation 25 mg/kg artequin

TABLE 5
Biological parameters in the two groups at Day 0 and Day 7

Parameters	AS + MQ			AL		
	<i>N</i> = 160			<i>N</i> = 160		
	Day 0	Day 7	<i>P</i>	Day 0	Day 7	<i>P</i>
Mean Hb* level (g/dL)	9.6	9	NS	10.1	9.2	NS
Anemia (Hb < 11 g/dL) (%)	60.6	65.6	0.35	51.9	73.7	< 10 ⁻³
Mean ALAT* level	31.9	19.8	0.006	28.4	18.5	0.004
Patients with normal ALAT (< 40 UI/L) (%)	70.6	90	< 10 ⁻³	82.5	94.3	8.10 ⁻³
Mean ASAT* level	51.3	33.4	0.001	48.4	31.1	< 10 ⁻³
Patients with normal ASAT (< 40 UI/L)	40.6	68.7	< 10 ⁻³	48.1	80	< 10 ⁻³
Mean bilirubine	8.7	4.5	0.002	8.8	5.2	< 10 ⁻³
Mean creatinine level	6.2	6	NS	6.5	6.4	NS

*Hb = haemoglobin; ALAT = alanine aminotransferase; ASAT = aspartate amino transferase.

pediatric has been adapted in Africa for the most vulnerable group to malaria.

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REFERENCES

1. World Health Organization, 2006. *Guidelines for the Treatment of Malaria*. Geneva: World Health Organization. WHO/HTM/RBM 2006.1108.
2. Massougboji A, Kone M, Kinde-Gazard D, Same-Ekobo A, Cambon N, Mueller EA, 2002. A randomized, double-blind study on the efficacy and safety of a practical three-day regimen with artesunate and mefloquine for the treatment of uncomplicated *Plasmodium falciparum* malaria in Africa. *Trans R Soc Trop Med Hyg* 96: 655–659.
3. Agomo PU, Meremikwu MM, Wtila IM, Omalu II, Odey FA, Oguiche S, Ezeiru VI, Aina OO, 2008. Efficacy, safety and tolerability of artesunate-mefloquine in the treatment of uncomplicated *Plasmodium falciparum* malaria in four geographic zones of Nigeria. *Malar J* 7: 172.
4. Ndiaye JL, Faye B, Diouf A, Kuété T, Cisse M, Seck PA, Brasseur P, Same-Ekobo A, Lameyre V, Gaye O, 2008. Randomized, comparative study of the efficacy and safety of artesunate plus amodiaquine, administered as a single daily intake versus two daily intakes in the treatment of uncomplicated falciparum malaria. *Malar J* 7: 16.
5. World Health Organization, 2000. *Management of Severe Malaria: A Practical Handbook*. Second Edition. Geneva: WHO.
6. World Health Organization, 1999. *Management of Severe Malnutrition: A Manual for Physicians and Other Senior Health Workers*. Geneva: WHO.
7. World Health Organization, 2003. *Assessment and Monitoring of Antimalarial Drug Efficacy for the Treatment of Uncomplicated Falciparum Malaria*. Geneva: World Health Organization. WHO/HTM/RBM/2003.50.
8. Faye B, Ndiaye JL, Dieng Y, Faye O, Gaye O, 2007. Efficacy and tolerability of four antimalarial combinations in the treatment of uncomplicated *Plasmodium falciparum* malaria in Senegal. *Malar J* 6: 80.
9. Sagara I, Dicko A, Djimde A, Guindo O, Kone M, Tolo Y, Thera MA, Sogoba M, Fofana M, Ouattara A, Sissoko M, Jansen HF, Doumbo OK, 2008. A randomized trial of artesunate-mefloquine versus artemether-lumefantrine for the treatment of uncomplicated *Plasmodium falciparum* malaria in Mali. *Am J Trop Med Hyg* 79: 655–661.
10. Valecha N, Srivastava P, Mohanty SS, Mittra P, Sharma SK, Tyagi PK, Pradhan K, Dev V, Singh R, Dash AP, Sharma YD, 2009. Therapeutic efficacy of artemether-lumefantrine in uncomplicated falciparum malaria in India. *Malar J* 8: 107.
11. Hutagalung R, Paiphun L, Ashley EA, McGready R, Brockman A, Thwai KL, Singhasivanon P, Jelinek T, White NJ, Nosten FH, 2005. A randomized trial of artemether-lumefantrine versus mefloquine-artesunate for the treatment of uncomplicated multi-drug resistant *Plasmodium falciparum* on the western border of Thailand. *Malar J* 4: 46.
12. Nosten F, van Vugt M, Price R, Luxemburger C, Thway KL, Brockman A, McGready R, ter Kuile F, Looaresuwan S, White NJ, 2000. Effects of artesunate-mefloquine combination on incidence of *Plasmodium falciparum* malaria and mefloquine resistance in western Thailand: a prospective study. *Lancet* 356: 297–302.