MAJOR ARTICLE

Efficacy of Artesunate Plus Amodiaquine versus That of Artemether-Lumefantrine for the Treatment of Uncomplicated Childhood *Plasmodium falciparum* Malaria in Zanzibar, Tanzania

Andreas Mårtensson,[1,3,a] Johan Strömberg,[1,a] Christin Sisowath,[1] Mwinyi I. Msellem,[5] J. Pedro Gil,[1] Scott M. Montgomery,[2,4] Piero Olliaro,[6] Abdullah S. Ali,[5] and Anders Björkman[1]

[1]Infectious Diseases Unit, Department of Medicine, and [2]Clinical Epidemiology Unit, Karolinska University Hospital, Karolinska Institute, Stockholm, [3]Emergency Medicine Unit, Department of Medicine, Kullbergska Hospital, Katrineholm, and [4]Clinical Research Center, Örebro University Hospital, Örebro, Sweden; [5]Malaria Control Program, Ministry of Health and Social Welfare, Zanzibar, Tanzania; and [6]World Health Organization Special Program for Research and Training in Tropical Diseases, Geneva, Switzerland

(See the editorial commentary by Whitty and Staedke on pages 1087–8)

Background. This is the first clinical trial comparing the efficacy of artesunate plus amodiaquine (ASAQ) and artemether-lumefantrine (AL)—the major artemisinin-based combination therapy (ACT) candidates for treatment of malaria in Africa—that involved an extended, 42-day follow-up period, polymerase chain reaction–adjusted parasitological cure rates (PCR APCRs), and systematic analyses of genetic markers related to quinoline resistance.

Methods. A total of 408 children with uncomplicated *Plasmodium falciparum* malaria in Zanzibar, Tanzania, were enrolled. Children who were 6–8 months of age and/or who weighed 6–8 kg were assigned to receive ASAQ for 3 days. Children who were 9–59 months of age and who weighted ⩾9 kg were randomly assigned to receive either ASAQ or AL for 3 days in standard doses. Intention-to-treat analyses were performed.

Results. Age- and weight-adjusted PCR-APCRs by follow-up day 42 were 91% (188 of 206 patients) in the ASAQ group and 94% (185 of 197 patients) in the AL group (odds ratio [OR] for the likelihood of cure, 2.07; 95% confidence interval [CI], 0.84–5.10; $P = .115$). A total of 5 and 7 recrudescences occurred after day 28 in the ASAQ and AL groups, respectively. On the assumption that 10 malaria episodes with uncertain PCR results were recrudescences, PCR-APCRs decreased to 88% in the ASAQ group and to 92% in the AL group. Unadjusted cure rates by day 42 were 56% (116 of 206 patients) in the ASAQ group versus 77% (151 of 197 patients) in the AL group (OR, 2.55; 95% CI, 1.66–3.91; $P < .001$). Rates of reinfection by day 42 were 36% (65 of 181 patients) in the ASAQ arm versus 17% (31 of 182 patients) in the AL arm (OR, 0.37; 95% CI, 0.22–0.60; $P < .001$). A significant selection of *P. falciparum* multidrug resistance gene 1 allele 86N was found in isolates associated with reinfection after AL treatment, compared with isolates at baseline (2.2-fold increase; $P < .001$).

Conclusions. Both treatments were highly efficacious, but AL provided stronger prevention against reinfection. The high proportion of recrudescences found after day 28 and the genetic selection by the long-acting partner drug underlines the importance of long follow-up periods in clinical trials. A long follow-up duration and performance of PCR genotyping should be implemented in programmatic surveillance of antimalarial drugs.

Because of the emerging resistance of *Plasmodium falciparum* to conventional antimalarial drugs, sub-Saharan Africa stands at the edge of a paradigm shift in the treatment of uncomplicated malaria. Artemisinin-based combination therapy (ACT) is increasingly being advocated as the way forward [1]. The concept of ACT is based on the use of 2 drugs with different modes of action: an artemisinin-derivative that causes rapid and effective reduction of parasite biomass and gametocyte carriage and a partner drug that has a longer duration of action. The hypothesis is that these 2 drugs together will achieve effective clinical and parasitological cure, protect each other from the development of resistance by *P. falciparum,* and reduce the overall rate of malaria

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[a] A.M. and J.S. contributed equally to this work.

Reprints or correspondence: Dr. Andreas Mårtensson, Infectious Diseases Unit, Dept. of Medicine, Karolinska University Hospital, 171 76 Stockholm, Sweden (andreas.martensson@medks.ki.se).

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transmission. Proof of the principle of ACT has so far only been demonstrated in Southeast Asia with the combination of artesunate and mefloquine [2].

In Africa, current evidence on the efficacy and safety of ACT is limited, but 3 ACTs are considered to be realistic for widespread use: artemether-lumefantrine (AL), artesunate plus amodiaquine (ASAQ), and artesunate plus sulfadoxine-pyrimethamine [3]. The quinoline partner drugs in AL (lumefantrine [aryl-amino alcohol]) and ASAQ (amodiaquine [4-aminoquinoline], through its main active metabolite desetyl-amodiaquine) both have long elimination half-lives estimated to be 4–10 days [4, 5]. Because the artemisinin-derivatives are rapidly eliminated, the slowly eliminated partner drug remains unprotected in subtherapeutic concentrations for a considerable period.

Recent 28-day follow-up studies in Africa comparing AL and ASAQ with conventional monotherapies have shown superior efficacy and good tolerability of the ACTs [6–10]. However, only 1 efficacy trial comparing AL and ASAQ has been published, but it had a short follow-up duration of 14 days and lacked PCR-adjusted cure rates [11].

The duration of follow-up in efficacy trials is critical, and to avoid underestimating treatment failure rates, the new World Health Organization guideline recommends 42 days of follow-up for patients treated with lumefantrine [12]. PCR genotyping is required in clinical trials performed in areas where the malaria transmission rate is high, to distinguish infection with parasites that have escaped drug action (i.e., recrudescent infection) from infection with parasites associated with new inoculation (i.e., reinfection) [13].

In November 2001, the Zanzibar, Tanzania, Ministry of Health and Social Welfare decided that Zanzibar would be one of the first regions in Africa to replace chloroquine with ASAQ and sulfadoxine-pyrimethamine with AL as first-line and second-line treatments, respectively. We report baseline data before implementation of the new drug policy on the efficacy, safety, and possible selection of markers of resistance to ASAQ and AL during an extended follow-up period of 42 days in a clinical trial conducted between November 2002 and February 2003 involving 408 Zanzibar children with uncomplicated *P. falciparum* malaria.

PATIENTS AND METHODS

Study design, sites, and patients. This comparative clinical trial of the efficacy and tolerability of ASAQ versus that of AL for treatment of uncomplicated childhood *P. falciparum* malaria was conducted at the following 2 sites in Zanzibar: Kivunge Hospital, Unguja Island, and Micheweni Hospital, Pemba Island. Both hospitals are located in densely populated rural areas with holoendemic *P. falciparum* transmission. At the time of the study, the local government supplied chloroquine and sulfadoxine-pyrimethamine to the sites. Other antimalarial drugs—but no ACTs—were available in the private sector.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Zanzibar Health Research Council and by the ethics committee at Karolinska Institutet (Stockholm, Sweden). Written informed consent was obtained from the parents or legal guardians of enrolled children.

Study participants were recruited among patients presenting at the outpatient departments of the hospitals. At the time the study was conducted, AL was not registered for use in children who were <9 months of age or weighed <9 kg, whereas there were no such limitations for ASAQ. This necessitated minor adjustments of age-based and weight-based enrollment criteria associated with the 2 treatments.

Inclusion criteria were as follows: age of 6–59 months and body weight of ⩾6 kg (for ASAQ) or age of 9–59 months and body weight of ⩾9 kg (for AL); parasitemia level of 2000–200,000 asexual parasites/μL of blood; and axillary temperature of ⩾37.5°C at the time of enrollment or history of fever during the preceding 24 h. Exclusion criteria were as follows: symptoms and/or signs of severe malaria, any danger sign (persistent vomiting; inability to sit, stand, drink or breastfeed; recent history of convulsions; and/or lethargy or otherwise impaired consciousness), hemoglobin concentration of <50 g/L, and serious underlying disease or known allergy to the study drugs.

Procedures. Enrolled patients were given an identity code and were assigned to receive a loose combination of artesunate (Plasmotrim 100 mg; Mepha S.A.) and amodiaquine (Flavoquin 153 mg; Roussel) or a fixed combination of 20 mg/120 mg AL (Coartem; Novartis). Children who were 6–8 months of age and/or had a body weight of 6–8 kg were assigned to receive ASAQ. Children who were 9–59 months of age and had a body weight of ⩾9 kg were randomly assigned to receive either of the 2 treatments.

ASAQ was administered as follows: 4 mg/kg body weight of artesunate plus 10 mg/kg body weight of amodiaquine once daily for 3 days. One tablet of AL was administered twice daily for 3 days to children with a body weight of 9 to <15 kg, and 2 tablets were administered twice daily for 3 days to children with a body weight of ⩾15 to 25 kg. All drugs were administered under direct observation. Full doses of drugs were readministered if a patient spat up or vomited within 30 min after receipt. Patients who vomited more than twice were excluded from the study.

The outpatient follow-up duration was 42 days. Clinical assessment and laboratory tests were performed on follow-up days 0, 1, 2, 3, 7, 14, 21, 28, 35, and 42 or on any day of recurrent illness. This included history of clinical symptoms, possible adverse events, concomitant drug consumption, clinical examination, and measurement of axillary temperature. Clinical data were recorded on the case record form. If a patient

received a diagnosis of malaria after day 14, this was considered to be a new episode and was treated accordingly. If, however, treatment failure occurred within 14 days or if severe malaria was diagnosed, rescue treatment with quinine was initiated.

Giemsa-stained thick blood films were examined by experienced microscopists during each visit. Parasitemia was quantified by a standard approximation method (40 × the number of parasites per 200 leukocytes on thick film). A positive smear result was defined as the presence of at least 1 asexual parasite seen during examination of 100 thick-smear fields under ×1000 magnification. Quality control by means of independent examination in a central laboratory was done for 10% of the slides.

Hemoglobin levels were measured by means of the HemoCue B-Hemoglobin Photometer on days 0, 3, 7, 14, 21, 28, 35, and 42 or on the days of recurring fever and/or parasitemia. Total and differential WBC counts were manually counted using a standard procedure on days 0, 3, 7, 14, 21, and 28 [14]. Blood samples were collected and spotted onto filter papers (3MM; Whatman) for parasite genotyping on days 0, 3, 7, 14, 21, 28, 35, and 42 or on the day of recurrent fever and/or parasitemia.

Molecular analyses. PCR genotyping of the gene encoding merozoite surface protein 2 (*msp2*), considered to be the most informative single genetic marker for multiplicity of infection of *P. falciparum,* was performed to differentiate reinfection from recrudescence [15]. Paired PCR results were analyzed between day 0 and the day of recurrent parasitemia (from day 14 up to day 42) by use of a standard protocol [16]. Single-nucleotide polymorphisms of *P. falciparum* multidrug resistance gene 1 (*pfmdr1*) N86Y and *P. falciparum* chloroquine resistance transporter gene (*pfcrt*) K76T were analyzed according to established *Apo*I restriction enzyme–based PCR-RFLP protocols [17].

Outcomes. The primary outcome was the PCR-adjusted parasitological cure rate up to day 42 of the follow-up period. The standard World Health Organization classification of outcomes—early treatment failure, late clinical failure, late parasitological failure, and adequate clinical and parasitological response by day 14—was also used. The clinical and parasitological response outcome was extended beyond day 14 (i.e., cure rates were recorded on days 28 and 42) and defined as the absence of both recurrent parasitemia and clinical symptoms suggestive of severe malaria.

Parasitological cure was adjusted using PCR genotyping of *msp2*. Recrudescence was defined as the presence of at least 1 matching allelic band, and reinfection was defined as the absence of any matching allelic band on day 0 and on the day of parasitemia recurrence. Patients who had recurrent parasitemia and no blood sample on filter paper or negative PCR results were considered to have an uncertain PCR-adjusted outcome. Samples showing infections with mixed *pfcrt* K76T and *pfmdr1* N86Y single-nucleotide polymorphisms were considered to be double contributors to the allele frequency.

Drug tolerability and safety were assessed clinically and by means of laboratory tests. An adverse event was defined as any undesirable medical occurrence (symptom, sign, or laboratory finding) in a patient during the study regardless of whether it was related to the treatments. Adverse events were judged in the field by clinicians according to their causal association with ACT (unlikely, possible, and probable) and severity (mild, moderate, or severe). Withdrawal due to clinically suspected severe malaria was regarded clinical treatment failures. Additional secondary outcomes included parasite and fever clearance and possible selection of drug resistance single-nucleotide polymorphisms.

Statistical analyses. Sample size was calculated such that an efficacy rate of 85% could be estimated with 95% CIs with intervals of ≤10%. To achieve this level of precision, 196 patients were required in each arm after losses due to attrition, which were estimated to be 10% of the initial number of patients enrolled.

Data were entered, validated, and analyzed using SPSS software, version 11.5 (SPSS). Primary and secondary outcomes for all patients in each treatment arm were analyzed by means of intention-to-treat analysis. Data for patients who were lost to follow-up without a defined primary efficacy outcome were kept in the analyses until the day of exit.

Proportions were compared using the χ^2 test or Fisher's exact test, as appropriate. Continuous variables were compared using Student's *t* test. Statistical significance was defined as a *P* value of ≤.05. Multiple logistic regression analyses were performed for all primary outcomes, using series of binary dummy variables to adjust for differences in age and weight inclusion criteria. The following variables were incorporated into the logistic regression analyses: treatment, study site, sex, age group (6–8 months, 9–17 months, 18–23 months, 24–35 months, or ≥36 months), weight group (4 to <9 kg, 9 to <11 kg, 11 to <13 kg, 13 to <15 kg, or ≥15 kg), and a final variable consisting of the combination of age of <9 months and weight of <9 kg (which represented children who could only be assigned to the ASAQ arm). Two separate analyses were performed for the group of patients who had uncertain PCR-adjusted outcomes; such outcomes were defined as either recrudescences or reinfections to estimate the range of possible PCR-adjusted parasitological cure rates.

RESULTS

Patient characteristics and flow through the study. A total of 2097 children were screened for study eligibility, and 1689 did not meet the criteria for enrollment. Of the 408 patients who were enrolled (208 in the ASAQ arm and 200 in the AL arm), 303 were from Kivunge, and 105 were from Micheweni. Demographic, clinical, and laboratory characteristics at the time of enrollment are presented in table 1. Figure 1 shows the flow

Table 1. Characteristics of patients at enrollment who received artesunate-amodiaquine (ASAQ) or artemether-lumefantrine (AL) for treatment of malaria.

Characteristic	ASAQ group (n = 207)	AL group (n = 200)
Sex, no. of patients		
Male	104	107
Female	103	93
Age, months, median (range)	24.3 (4.8–73.3)	34.9 (6.0–61.6)
Weight, kg, median (range)	10.2 (6.0–20.0)	12.2 (9.0–21.0)
Parasite count, parasites/μL, geometric mean (range)	19,731 (2000–198,440)	13,731 (2000–200,000)
Temperature, °C, arithmetic mean ± SD	38.7 ± 1.2	38.7 ± 1.2
Hemoglobin level, g/L, arithmetic mean ± SD	85 ± 16	87 ± 15
Leukocyte count, cells/mm^3, arithmetic mean ± SD	6564 ± 2646	6106 ± 2905
Neutrophil count, cells/mm^3, arithmetic mean ± SD	3510 ± 2128	3273 ± 2110

of patients through the trial. One patient assigned to receive ASAQ was excluded because the slide obtained on day 0 showed no organisms when rechecked. Patients with minor violations of age or weight inclusion criteria were not excluded. A total of 403 (99%) of 407 children complied freely with the study protocol up to the time of a new malaria episode or follow-up day 42. Four children were lost to follow-up; 1 child in the ASAQ group and 2 in the AL group were withdrawn at the guardian's request, and 1 in the AL group migrated out of the study area.

Adequate clinical and parasitological response by follow-up day 14 and cure rates on follow-up days 28 and 42. Non–PCR-adjusted adequate clinical and parasitological response rates by follow-up day 14, cure rates on follow-up days 28 and 42, and ORs for the likelihood of cure are presented in table 2. Three children (1 in the ASAQ arm and 2 in the AL arm) developed early treatment failure. Non–PCR-adjusted late parasitological failure and late clinical failure up to follow-up day 14 occurred in 2 and 3 children, respectively, all of whom were in the ASAQ group. Young age (<9 months) and low weight (<9 kg) were not associated with a decreased cure rate.

PCR-adjusted parasitological cure rates. PCR-adjusted parasitological cure rates associated with ASAQ and with AL, as well as corresponding ORs for likelihood of cure, are presented in table 2. Ten patients (7 in the ASAQ group and 3 in the AL group) had uncertain PCR-adjusted outcomes; 7 had no blood sample on filter paper, and 3 had negative PCR results.

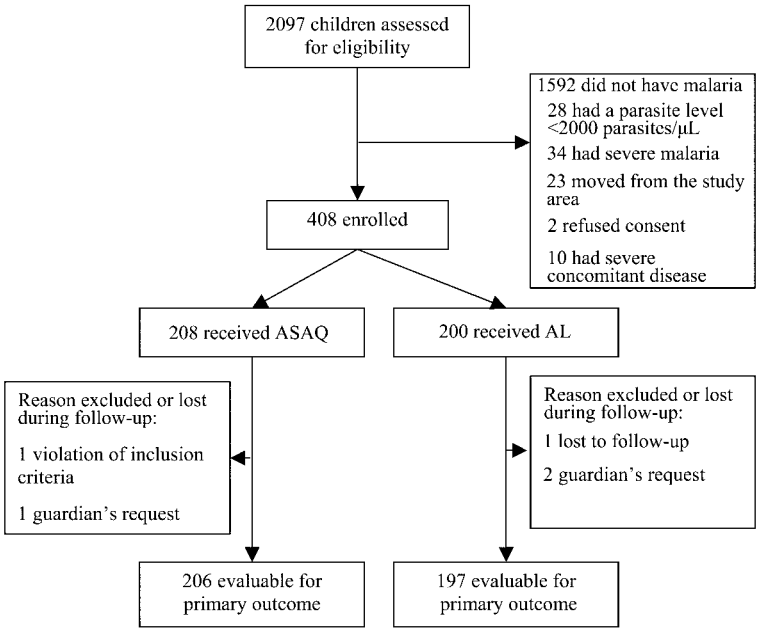

Figure 1. Flow of patients through the study. AL, artemether-lumefantrine; ASAQ, artesunate-amodiaquine.

Table 2. Falciparum malaria cure rates and likelihood-of-cure analyses associated with receipt of artesunate-amodiaquine (ASAQ) and artemether-lumefantrine (AL).

Cure rate, by follow-up day	Proportion (%) of patients who achieved cure, by treatment group		Likelihood-of-cure analysis			
			Unadjusted		Adjusted[a]	
	ASAQ	AL	OR (95% CI)	*P*	OR (95% CI)	*P*
Day 14						
Adequate clinical and parasitological	200/206 (97)	197/199 (99)	2.96 (0.59–14.82)	.188	7.37 (1.18–45.88)	.032
PCR adjusted						
Uncertain results defined as reinfections	203/206 (99)	197/199 (99)	1.46 (0.24–8.80)	.683	4.27 (0.59–30.81)	.150
Uncertain results defined as recrudescences	203/206 (99)	197/199 (99)	1.46 (0.24–8.80)	.683	4.27 (0.59–30.81)	.150
Day 28						
PCR unadjusted	149/206 (72)	183/197 (93)	5.00 (2.68–9.33)	<.001	5.70 (2.73–11.93)	<.001
PCR adjusted						
Uncertain results defined as reinfections	193/206 (94)	192/197 (97)	2.59 (0.90–7.40)	.076	5.40 (1.64–17.84)	.006
Uncertain results defined as recrudescences	188/206 (91)	192/197 (97)	3.68 (1.34–10.10)	.012	7.03 (2.16–22.83)	.001
Day 42						
PCR unadjusted	116/206 (56)	151/197 (77)	2.55 (1.66–3.91)	<.001	2.62 (1.56–4.41)	<.001
PCR adjusted						
Uncertain results defined as reinfections	188/206 (91)	185/197 (94)	1.48 (0.69–3.15)	.314	2.07 (0.84–5.10)	.115
Uncertain results defined as recrudescences	181/206 (88)	182/197 (92)	1.68 (0.86–3.28)	.132	2.32 (1.02–5.27)	.045

[a] Adjustment with logistic regression analyses was done for age, weight, treatment, sex, and study site.

A total of 5 (28%) of 18 cases of confirmed cases of recrudescence in the ASAQ group and 7 (58%) of 12 such cases in the AL group occurred after follow-up day 28. Similar to the findings of adequate clinical and parasitological response rates by follow-up day 14 and cure rates on follow-up days 28 and 42, young age and low weight were not associated with an increased risk of treatment failure; results of adjusted logistic regression analyses showed an increased association between the treatments, compared with results of unadjusted analysis.

Reinfection rates. Reinfection rates among children who achieved PCR-adjusted parasitological cure by day 42 were 36% for the ASAQ group (65 of 181 patients) and 17% for the AL group (31 of 182 patients), showing a significantly lower risk of reinfection after receipt of AL (unadjusted OR, 0.37; 95% CI, 0.22–0.60; $P < .001$).

Parasite and fever clearance. Data on the proportion of patients who achieved parasite and fever clearance during the 3 days of treatment are presented in figures 2 and 3, respectively. Day 1 slides with negative results were observed for 69 (34%) of 206 ASAQ-treated patients and for 33 (17%) of 199 AL-treated patients (unadjusted OR, 0.40; 95% CI, 0.25–0.63; $P < .001$). Absence of fever (defined as a temperature of <37.5°C) on day 1 was found for 162 (79%) of 205 ASAQ recipients and for 134 (67%) of 199 AL recipients (unadjusted OR, 0.55; 95% CI, 0.35–0.86; $P = .008$). ASAQ treatment was thus associated with more rapid clearance of parasites and fever.

Gametocyte carriage. Gametocyte carriage was similarly low in both treatment groups. At baseline, gametocyte carriage occurred in 2 of 207 patients in the ASAQ group and in 3 of 200 patients in the AL group. A relative peak was observed on day 1 in the ASAQ arm, with 8 gametocyte carriers. On day 7, only 5 children (4 in the ASAQ group and 1 in the AL group) had detectable gametocyte counts. After day 7, only 1 additional gametocyte carrier was found (on day 42).

Adverse events. Both treatment regimens were generally

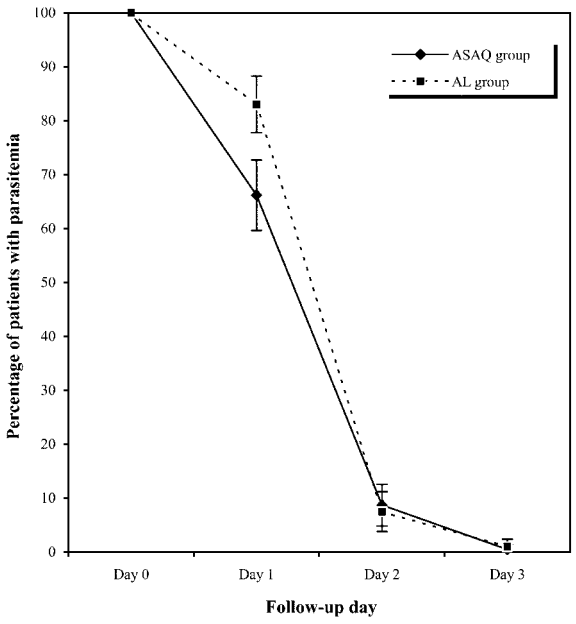

Figure 2. Parasite clearance during the first 3 days of the follow-up period, revealed as the percentage of patients with parasitemia. AL, artemether-lumefantrine; ASAQ, artesunate-amodiaquine. *Bars,* 95% CIs.

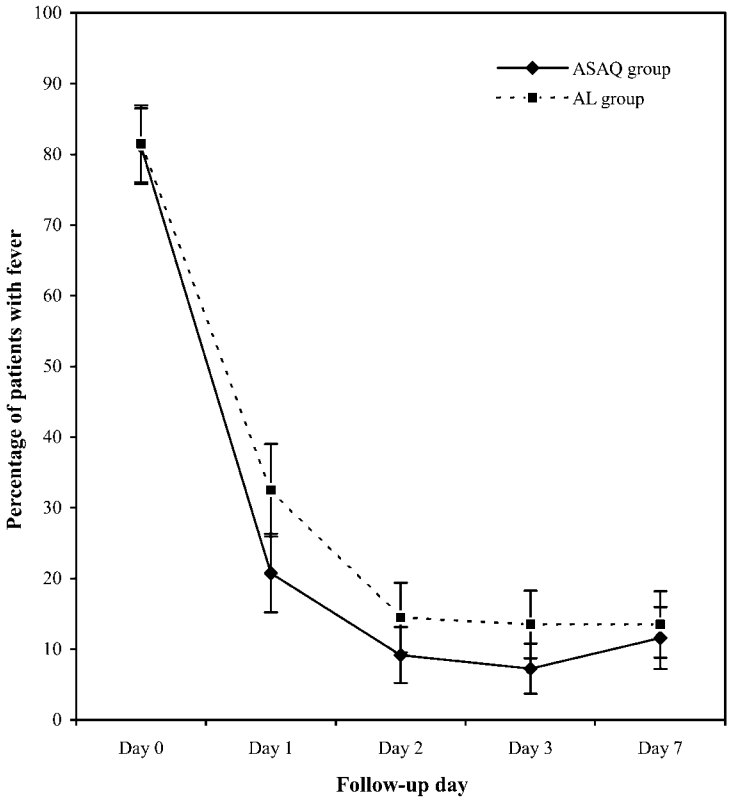

Figure 3. Fever clearance during the first 7 days of the follow-up period, revealed as the percentage of patients with a temperature of ⩾37.5°C. AL, artemether-lumefantrine; ASAQ, artesunate-amodiaquine. *Bars,* 95% CIs.

well tolerated. No deaths occurred, but 9 patients (7 in the ASAQ group and 2 in the AL group) developed clinically suspected severe malaria during the follow-up period and received rescue treatment. This difference was not statistically significant (unadjusted OR, 0.29; 95% CI, 0.06–1.41; $P = .124$). A severe or moderate adverse event was reported by 25 (12%) of 207 children treated with ASAQ and by 21 (10%) of 200 children treated with AL (unadjusted OR, 0.85; 95% CI, 0.46–1.58; $P = .616$). However, all 9 severe adverse events were associated with clinically suspected severe malaria and thus could not be attributed to the intervention drugs. No significant differences were seen in mean counts of WBCs and neutrophils between the 2 treatment groups during follow-up.

Recovery of the hemoglobin level. A significant and similar increase in the mean hemoglobin level from baseline to day 42 was noted in both intervention groups. Mean hemoglobin levels increased from 85 g/L (95% CI, 83–87 g/L) to 99 g/L (95% CI, 97–101 g/L) in the ASAQ group and from 87 g/L (95% CI, 85–89 g/L) to 102 g/L (95% CI, 100–104 g/L) in the AL group.

PCR-RFLP analyses of* pfcrt *K76T and* pfmdr1 *N86Y single-nucleotide polymorphisms. Baseline prevalences of *pfcrt* 76T and *pfmdr1* 86Y alleles were high: *pfcrt* 76T was detected in 383 (97%) of 396 *P. falciparum* isolates (95% CI, 94.5–98.2), and *pfmdr1* 86Y was detected in 335 (76%) of 443 *P. falciparum* isolates (95% CI, 71.6–79.6). After treatment, the relative frequency of *pfmdr1* 86Y decreased with a corresponding increase in the frequency of *pfmdr1* 86N among *P. falciparum* isolates from patients in the AL arm, whereas no such changes were observed among isolates from patients in the ASAQ arm (table 3). This increase was solely observed among *P. falciparum* isolates recovered during reinfection (2.2-fold increase; $\chi^2 = 11.824$; $P < .001$). The baseline frequency of *pfcrt* 76T was too high to allow any meaningful comparison.

DISCUSSION

Although artemisinin-based combination therapy (ACT) is generally recognized as a crucial instrument in the Roll Back Malaria Partnership's Global Strategic Program [18], comparisons of different ACTs are lacking. This study provides critically needed data about the relative efficacy and safety of 2 ACTs (ASAQ and AL), as well as data on their potential selection of genetic markers related to drug resistance. Zanzibar has opted for ASAQ as the new first-line treatment for uncomplicated *P. falciparum* malaria, whereas other countries have primarily chosen AL. Many African countries still have to make the critical

Table 3. Proportions of *Plasmodium falciparum* harboring *P. falciparum* multidrug resistance gene 1 (*pfmdr1*) 86N at baseline and during breakthrough infection.

Infection episode	Patients who received artemether-lumefantrine		Patients who received artesunate-amodiaquine	
	Proportion (%) of *P. falciparum* with *pfmdr1* 86N	95% CI, %	Proportion (%) of *P. falciparum* with *pfmdr1* 86N	95% CI, %
Baseline				
All patients	51/217 (23.5)[a]	17.9–29.1	57/226 (25.2)	19.6–30.9
Patients with recrudescence during follow-up[b]	3/13 (23.1)	5.0–53.8	3/20 (15.0)	3.2–37.9
Patients without nonrecrudescence during follow-up[b]	48/203 (23.6)	17.8–29.5	27/126 (21.4)	14.3–28.6
Breakthrough				
All patients	22/46 (47.8)[a]	32.9–63.1	18/95 (18.9)	11.6–28.3
Patients with recrudescence[b]	4/12 (33.3)	9.9–65.1	6/20 (30.0)	11.9–54.3
Patients with reinfection[b]	18/34 (52.9)[a]	35.1–70.2	12/75 (16.0)	8.6–26.3

NOTE. Mixed infections are considered to contribute 1 *pfmdr1* 86N allele and 1 *pfmdr1* 86Y allele.

[a] Statistically significant increase between baseline infection and breakthrough infection.

[b] *msp2* adjustment was performed to differentiate between recrudescence and reinfection..

decision about when to make the change and which ACT to choose. To facilitate decision making, policy makers need data from efficacy trials comparing major ACT candidates during adequate follow-up periods. Cost and drug formulations are other important factors influencing the choice of ACT. For example, the loose combination of ASAQ is still cheaper than the fixed AL formulation for treatment of adults (US$1.30 vs. US$2.40) [19].

A strength of this study lies in the long follow-up period of 42 days and the remarkably low rate of follow-up losses (403 [99%] of 408 enrolled children were evaluable for primary outcome), making it unique among efficacy trials in Africa. Multivariate analyses were performed to adjust for confounding due to age-based and weight-based differences in inclusion criteria stipulated in the licenses of the respective study drugs at the time of the trial. Because adjustment increased rather than decreased the unadjusted association between the treatments and each primary efficacy outcome, we are confident that our findings are not based on slight differences in inclusion criteria. Also, we observed similar findings when the analysis was restricted to children with the same age and weight characteristics from each treatment arm.

The handling and interpretation of uncertain PCR results represent a dilemma in clinical trials. We considered that patients with such results could have had recrudescence or reinfection and performed 2 separate analyses accounting for these possibilities. We therefore present the range of possible PCR-adjusted outcomes instead of fixed results. This increases the robustness of the analyses and neither underestimates nor overestimates the PCR-adjusted parasitological cure rate.

Both ASAQ and AL were well tolerated and highly efficacious. The PCR-adjusted parasitological cure rate on follow-up days 14 and 28 were higher or similar to findings in previously published studies on ASAQ and AL in Africa [6–11]. However, no previous day-42 efficacy data from Africa are available for comparison.

We found a significant proportion of recrudescences occurring between days 29 and 42, most prominently in the AL group. This finding substantiates the critical importance of a 42-day follow-up period, especially for patients who have received AL, to avoid underestimating true rates of treatment failure. This is in line with a recent meta-analysis performed in settings with low-to-medium malaria transmission, where assessments on day 28 missed 15% of the total treatment failures identified by day 42 [20], compared with misses of 28% (for ASAQ) and 58% (for AL) on day 28 in our study (which was possibly due to prolonged suppression of recrudescence by partially immune children).

The most striking difference between the 2 treatments was observed in the non–PCR-adjusted cure rates on days 28 and 42. This was largely a reflection of different reinfection rates, suggesting a longer period of protection against reinfection conferred by lumefantrine. However, prolonged elimination and protection intervals expose reinfecting parasites to low concentrations of the partner drug over a considerable period of time, which may drive the development of resistance to the drug in a setting of high malaria endemicity. An alarming finding, observed during episodes of reinfection in patients in the AL group, was therefore the selection of *pfmdr1* 86N, a marker previously associated with decreased susceptibility to lumefantrine in vitro [21, 22]. This may be viewed as a first step in a putative progressive accumulation of genetic alterations leading to future drug resistance against this quinoline [23]. The *pfcrt* 76T allele, which is associated with chloroquine resistance, was

detected in such a high frequency at baseline that further selection could not be assessed. However, the high frequency did not apparently affect treatment outcome with either drug.

In conclusion, this study provides unique efficacy data on follow–up day 42 for ASAQ and AL, which support their adequacy as first-line and second-line treatment, respectively, for uncomplicated malaria in Zanzibar, as well as their potential value for wider use in Africa. However, the molecular findings also suggest that ACTs are vulnerable to selection of genetic markers associated with drug resistance. Comparative clinical trials with long follow-up periods during which PCR genotyping and tests for detection of genetic markers related to drug resistance are performed should be implemented in programmatic surveillance of antimalarial drugs in Africa.

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References

1. White NJ, Nosten F, Looareesuwan S, et al. Averting a malaria disaster. Lancet **1999**; 353:1965–7.
2. Nosten F, van Vugt M, Price R, et al. Effects of artesunate-mefloquine combination on incidence of *Plasmodium falciparum* malaria and mefloquine resistance in western Thailand: a prospective study. Lancet **2000**; 356:297–302.
3. World Health Organization (WHO). Antimalarial drug combination therapy: report of a WHO Technical Consultation. Document no. WHO/CDS/RBM/2001.35. WHO: Geneva, Switzerland, **2001**.
4. Ezzet F, Mull R, Karbwang J. Population pharmacokinetics and therapeutic response of CGP (artemether + lumefantrine) in malaria patients. Br J Clin Pharmacol **1998**; 46:553–61.
5. Minzi OM, Rais M, Svensson JO, et al. High-performance liquid chromatographic method determination of amodiaquine, chloroquine and their monodesethyl metabolites in biological samples. J Chromatogr B **2003**; 783:473–80.
6. Adjuik M, Agnamey P, Babiker A, et al. Amodiaquine-artesunate versus amodiaquine for uncomplicated *Plasmodium falciparum* malaria in African children: a randomised multicentre trial. Lancet **2002**; 359: 1365–72.
7. Barennes H, Nagot N, Valea I, et al. A randomized trial of amodiaquine and artesunate alone and in combination for the treatment of uncomplicated falciparum malaria in children from Burkina Faso. Trop Med Int Health **2004**; 9:438–44.
8. von Seidlein L, Bojang K, Jones P, et al. A randomized controlled trial of artemether/benflumetol, a new antimalarial and pyrimethamine/sulfadoxine in the treatment of uncomplicated falciparum malaria in African children. Am J Trop Med Hyg **1998**; 58:638–44.
9. Hatz C, Abdulla S, Mull R, et al. Efficacy and safety of CGP 56697 (artemether and benflumetol) compared with chloroquine to treat acute falciparum malaria in Tanzanian children aged 1–5 years. Trop Med Int Health **1998**; 3:498–504.
10. Rwagacondo CE, Karema C, Mugisha V, et al. Is amodiaquine failing in Rwanda? Efficacy of amodiaquine alone and combined with artesunate in children with uncomplicated malaria. Trop Med Int Health **2004**; 9:1091–8.
11. Ndayiragije A, Niyungeko D, Karenzo J, et al. Efficacy of therapeutic combinations with artemisinin derivatives in the treatment of non complicated malaria in Burundi [in French]. Trop Med Int Health **2004**; 9:673–9.
12. World Health Organization (WHO). Assessment and monitoring of antimalarial drug efficacy for the treatment of uncomplicated falciparum malaria. Document no. WHO/HTM/RBM/2003.50. WHO: Geneva, Switzerland, **2003**.
13. Snounou GB, Beck H.-P. The use of PCR genotyping in the assessment of recrudescence or reinfection after antimalarial drug treatment. Parasitol Today **1998**; 14:462–7.
14. Dacie JV, Lewis SM. Practical haematology. 6th ed. Edinburgh: Churchill Livingstone, Longman Group, **1984**:38–40.
15. Farnert A, Arez AP, Babiker HA, et al. Genotyping of *Plasmodium falciparum* infections by PCR: a comparative multicentre study. Trans R Soc Trop Med Hyg **2001**; 95:225–32.
16. Snounou G, Zhu X, Siripoon N, et al. Biased distribution of *msp1* and *msp2* allelic variants in *Plasmodium falciparum* populations in Thailand. Trans R Soc Trop Med Hyg **1999**; 93:369–74.
17. Djimde A, Doumbo OK, Cortese JF, et al. A molecular marker for chloroquine-resistant falciparum malaria. N Engl J Med **2001**; 344: 257–63.
18. Roll Back Malaria Partnership. Roll back malaria. Available at: http://www.rbm.who.int/cgi-bin/rbm/rbmportal/custom/rbm/home.do#top. Accessed 24 August 2005.
19. Snow RW, Eckert E, Teklehaimanot A. Estimating the needs for artesunate-based combination therapy for malaria case-management in Africa. Trends Parasitol **2003**; 19:363–9.
20. Stepniewska K, Taylor WR, Mayxay M, et al. In vivo assessment of drug efficacy against *Plasmodium falciparum* malaria: duration of follow-up. Antimicrob Agents Chemother **2004**; 48:4271–80.
21. Duraisingh MT, Roper C, Walliker D, Warhurst DC. Increased sensitivity to the antimalarials mefloquine and artemisinin is conferred by mutations in the *pfmdr1* gene of *Plasmodium falciparum*. Mol Microbiol **2000**; 36:955–61.
22. Duraisingh MT, Jones P, Sambou I, von Seidlein L, Pinder M, Warhurst DC. The tyrosine-86 allele of the *pfmdr1* gene of *Plasmodium falciparum* is associated with increased sensitivity to the anti-malarials mefloquine and artemisinin. Mol Biochem Parasitol **2000**; 108:13–23.
23. Hastings IM. The origins of antimalarial drug resistance. Trends Parasitol **2004**; 20:512–8.