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Low efficacy of the combination artesunate plus amodiaquine for uncomplicated falciparum malaria among children under 5 years in Kailahun, Sierra Leone

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Summary

OBJECTIVE In 2004, Sierra Leone adopted artesunate plus amodiaquine as first-line antimalarial treatment. We evaluated the efficacy of this combination in Kailahun, where a previous study had shown 70.2% efficacy of amodiaquine in monotherapy.

METHODS Method and outcome classification of the study complied with WHO guidelines. Children 6–59 months with uncomplicated malaria were followed-up for 28 days. PCR genotyping was used to distinguish recrudescence from reinfection. Reinfections were reclassified as cured.

RESULTS Of 172 children who were referred to the study clinic, 126 satisfied inclusion criteria and were enrolled. No early treatment failures were reported. The day 14, efficacy was 98.2% (95% CI: 93.8–99.8). Of 65 recurrent parasitaemias analysed by PCR, 17 were recrudescences. The PCR-adjusted day 28 efficacy was 84.5% (95% CI: 76.4–90.7). All true failures occurred in the last 8 days of follow-up. Of 110 children who completed the 28-day follow-up, 54 (49.1%) experienced a novel infection.

CONCLUSION The efficacy of this combination was disappointing. The high reinfection rate suggested little prophylactic effect. In Kailahun a more efficacious combination might be necessary in the future. The efficacy of AS + AQ needs to be monitored in Kailahun and in the other regions of Sierra Leone.

keywords *Plasmodium falciparum*, artemisinin combination therapies, artesunate, amodiaquine, *in vivo* efficacy, Sierra Leone

Introduction

In 2002–2003, a multicentric antimalarial efficacy study was carried out in Sierra Leone by Checchi and colleagues (2005). This study demonstrated the low overall efficacy of both first- and second-line antimalarial drugs, chloroquine (CQ) and sulfadoxine-pyrimethamine (SP). The efficacy of amodiaquine (AQ), the third monotherapy tested, varied within the country, ranging from 94.6% to 70.2%. The lowest efficacy was reported in Kailahun (Checchi *et al.* 2005).

In 2004, the Ministry of Health and Sanitation, following WHO recommendations (WHO/RBM 2003), adopted artesunate plus AQ (AS + AQ) as first-line antimalarial treatment and artemether plus lumefantrine (Coartem®) as second-line. AS + AQ was expected to be highly efficacious (Adjuik *et al.* 2002) and well tolerated (Adjuik *et al.* 2004). However, the high efficacy of AS + AQ, that would expect with the introduction of a new treatment, could not be guaranteed in Kailahun, given the relatively low efficacy of monotherapy AQ in the previous year. In 2004 we assessed, therefore, the *in vivo* efficacy of AS + AQ in Kailahun. We report here the study results.

Methods and materials

The study took place at the outpatient department of Médecins Sans Frontières (MSF) hospital in Kailahun town (Figure 1). This structure also hosted the previous study and we followed the same protocol (Checchi *et al.* 2005), as summarized below.

Eligibility for inclusion and clinical and parasitological evaluation were according to WHO recommendations (WHO 1996). Children aged 6–59 months with confirmed uncomplicated *Plasmodium falciparum* malaria and meeting inclusion criteria were enrolled, treated on site with 3-day artesunate, 4 mg/kg/day (Arsumax®, Sanofi Winthrop AMO, Gentilly, France), and amodiaquine, 10 mg/

Figure 1 1 Location of study site.

kg/day (Camoquin®, Pfizer, Dakar, Senegal), and were followed-up for a period of 28 days.

Based on available data on the gain in efficacy when AS is added to AQ, and the known failure of AQ in Kailahun (29.8%), 15% failure was hypothesized for AS + AQ. A size of 126 children was estimated; assuming a 95% significance level (precision of ± 7.5%); taking in account 20% possible withdrawals; and, 20% expected reinfections occurring during follow-up.

Both thick and thin blood smears were taken from enrolled patients on day 0, 2, 3, 7, 14, 21, 28 and any other day if needed. Microscopic examination was done under 100× oil immersion magnification. A physician examined the child's clinical condition during the entire follow-up period.

All therapeutic failures received a rescue treatment consisting of oral quinine hydrochloride, 8 mg/kg every 8 h for 7 days, or artemether intramuscular, 3.2 mg/kg loading dose, followed by 1.6 mg/kg/day for at least 3 days, when oral intake was not possible. All children received anti-helmintic (mebendazole 250 mg or albendazole 400 mg). Children with initial haemoglobin below 10 g/dl received ferrous sulphate 30 mg/kg/day for 2 months and folic acid 5 mg/day, starting from day 7 for 1 month.

Gametocyte carriage was measured on days 0, 3, 7, 14, 21 and 28. Haemoglobin levels were measured on day 0, 14 and 28 with the Lovibond undiluted colorimetric technique (Lovibond, Assistant Co. Sondheim Rhon, Germany). Children were considered non anaemic when haemoglobin was ≥ 11.0 g/dl, mildly anaemic when haemoglobin was ≥8 and <11.0 g/dl, and moderately anaemic when haemoglobin was ≥5 and <8.0 g/dl.

PCR genotyping, using *msp-1* and *msp-2* markers, was applied to distinguish true recrudescences, due to treatment failure, from episodes of reinfection (WHO 2002). Recrudescence was identified when pre- and post-treatment sample alleles were the same; re-infection was identified when pre- and post-treatment sample alleles were distinct; and mixed recrudescence and re-infection when similar pre- and post-treatment samples alleles were found, but with additional distinct alleles identified. Analysis was carried out at the Laboratory of Parasitology and Mycology of Avicenne Hospital, Bobigny (France), using a published method (Ranford-Cartwright *et al.* 1997; Snounou *et al.* 1999).

Data were double entered and validated using EpiData (The EpiData Association, Odense, Denmark) and analysed using Stata 8.0 (College Station, TX). Total number of failures (early treatment failure + late clinical failure + late parasitological failure [ETF + LCF + LPF]) were calculated over the total number of analysable outcomes, and expressed as a percentage with associated 95% confidence intervals. During the 28-day follow-up, treatment failures, which were confirmed to be recrudescence or mixed recrudescence/reinfection by PCR genotyping, were classified as true failures, while pure reinfections were reclassified as adequate clinical and parasitological response (ACPR). Although the reclassification of reinfection as ACPR was no longer recommended (WHO 2003), we kept this method to compare our results with those of the previous multi-centric study (Checchi *et al.* 2005). Patients for which the PCR result was inconclusive (indeterminate or no DNA isolated), secondary exclusions and patients lost to follow-up were excluded from the analysis.

The failure proportion on day 14 (without PCR adjustment) was also calculated. Haemoglobin levels on day 0 and day 28 were compared (McNemar's test).

The Ethics Committee of the Ministry of Health and Sanitation of Sierra Leone approved the study protocol.

Results

Between 14 July and 26 August 2004, of 172 children aged 6–59 months admitted to the clinic, 126 satisfied the inclusion criteria, and were enrolled in the study. The male/female ratio was 0.75 (54/72). Other baseline characteristics are described in Table 1. Three children, although able to drink or breast feed, did not complete the treatment because of repeated vomiting of the drugs; 10 children were also withdrawn because of a concomitant illness requiring hospitalization ($n = 8$) or because of other

antimalarial intake ($n = 2$). Sixty-five children were found parasitaemic during follow-up (2 before and 63 after day 14) requiring PCR genotyping. Of these, one sample had no DNA amplified; 17 were treatment failures (10 simple recrudescence and 7 mixed recrudescences and reinfections); and 47 were pure reinfections. A novel infection (considering mixed recrudescence/reinfection and pure reinfection) was detected in 49.1% of children (54/110) (Table 2).

Day 14 therapeutic efficacy was 98.2% (112/114, 95% CI: 93.8–99.8) (Table 3), while day 28 PCR-adjusted therapeutic efficacy was 84.5% (93/110, 95% CI: 76.4–90.7). All true failures occurred in the last 8 days of follow-up. No early treatment failures were reported.

Fever clearance on day 2 (<37.5 °C) and parasite clearance on day 3 were 95.2% (119/125) and 94.2% (114/121) respectively. Haemoglobin recovery was assessed among the 87 children who attended for their day 28 visit. Among the 80 children anaemic on day 0 (71 mildly and 9 moderately anaemic), 27 (33.7%) were no longer anaemic on day 28 ($P < 0.001$), regardless of the therapeutic outcome; four children not anaemic on day 0 became moderately anaemic on day 28.

Table 1 Patient characteristics at inclusion

Age (months), median (IQR)	17	10–24
Gender ratio (M/F)	0.75	54/72
Asexual parasite density (/μl), geometric mean (IQR)	27 116	10 630–68 870
Parasitaemia > 10 000/μl, *n* (%)	96	76.2
Temperature (°C), median (IQR)	39.0	38.3–39.4
MUAC, median (IQR)	148	142–156
Haemoglobin, *n* (%)		
No anaemia (Hb ≥ 11 g/dl)	12	9.5
Mild anaemia (Hb 8–10.9 g/dl)	99	78.6
Moderate anaemia (Hb 5–7.9 g/dl)	15	11.9

IQR, Inter-quartile range; MUAC, middle-upper arm circumference.

Table 2 PCR genotyping results

	$n = 64$	
	n	%
Total PCR result available		
Recrudescence (A)	10	15.6
Mixed recrudescence and reinfection (B)	7	10.9
Reinfection (C)	47	73.4
Total confirmed treatment failure (A + B)	17	26.6
Total reinfection (B + C)	54	

Table 3 Day 14 and PCR-adjusted day 28 therapeutic responses

	Day 14 ($n = 114$)			Day 28 ($n = 110$)		
Outcomes	*n*	%	95% CI	*n*	%	95% CI
ETF	0	0.0	0.0–3.2*	0	0.0	0.0–3.3*
LCF	0	0.0	0.0–3.2*	9	8.2	3.8–15.0
LPF	2	1.8	0.2–6.2	8	7.3	3.2–13.8
Total failures	2	1.8	0.2–6.2	17	15.5	9.3–23.6
ACPR	112	98.2	93.8–99.8	93	84.5	76.4–90.7

*One-side, 97.5% CI; ETF, Early Treatment Failure; LCF, Late Clinical Failure, LPF, Late Parasitological Failure; ACPR, Adequate Clinical and Parasitological Response.

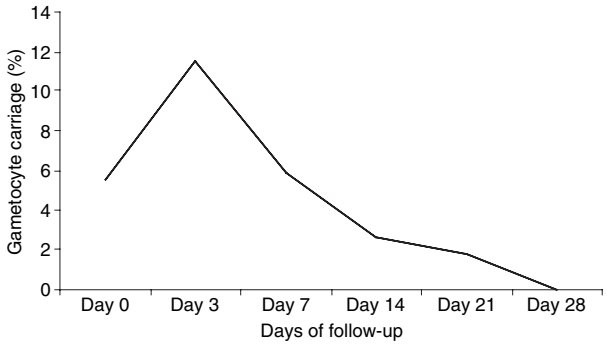

Figure 2 Proportion of children with gametocytes on different days of follow-up.

On the day of inclusion, 5.6% of children (7/126) were reported having gametocytes. The proportion increased to 11.6% (14/121) on day 7 and progressively decreased (Figure 2) until day 28 when none of the children were gametocytaemic.

Discussion

The results until day 14 were extremely promising. AS + AQ resulted in a high day 2 fever clearance and a high day 3 parasite clearance, which has been shown in previous studies (Staedke *et al.* 2004; Hamour *et al.* 2005). No early treatment failures were reported and therapeutic efficacy on day 14 was also very high (98.2%). The day 28 efficacy (84.5%), nevertheless, confirmed expectations and fears, given the already low efficacy of the monotherapy AQ the previous year (Checchi *et al.* 2005). These results questioned whether AS + AQ is suitable first-line treatment in Kailahun. Low efficacies of this combination have also been recently reported in Africa (Rwagacondo *et al.* 2004) and in Asia (Durrani *et al.* 2005).

On one hand, AS + AQ would undoubtedly contribute to a general improvement of malaria case management,

compared to the previous situation where malaria patients were treated with ineffective drugs. The day 28 failures were almost half of those that occurred with monotherapy AQ (15.5% *vs.* 29.2%) (Checchi *et al.* 2005); on day 28 no children were reported carrying gametocytes; and a considerable number reported improved haemoglobin level, even if this was probably the result of the combined effect of having recovered from malaria and the benefit of antihelmintic and supportive treatments.

On the other hand, the day 28 efficacy was far from what we would expect from a newly introduced treatment, and the high reinfection rate suggested that this combination provided little prophylactic effect. The maintenance of the current efficacy over time needs to be monitored and we cannot exclude that an alternative treatment might be necessary in the near future in Kailahun. Alternatives, however, are few. Artemether plus lumefantrine (Coartem®), the current second-line treatment, is highly efficacious in Africa (Mutabingwa *et al.* 2005; Piola *et al.* 2005), but it is currently more expensive than AS + AQ. Little information is yet available about the long term effects of the large scale use of ACTs in Africa. It is hoped that, as in Thailand (Brockman *et al.* 2000), the use of a drug in combination with an artemisinin slows the resistance process. The long-term positive results in Asia, though obtained in lower transmission settings, are encouraging. Further studies to monitor the efficacy of AS + AQ are necessary in Kailahun and in other regions of the country.

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Faible efficacité de la combinaison artésumate plus amodiaquine dans le traitement de la malaria non compliquée chez les enfants de moins de 5 ans à Kailahun en Sierra Leone

OBJECTIFS En 2004, la Sierra Leone a adopte la combinaison artésunate + amodiaquine comme traitement de première ligne de la malaria. Nous avons évalué l'efficacité de cette combinaison à Kailahun, où une précédente étude avait démontré une efficacité de 70,2% pour l'amodiaquine en monothérapie.
MÉTHODES Méthode et classification des résultats ont été réalisées selon les directives de l'OMS. Les enfants de 6 à 59 mois avec une malaria non compliquée ont été suivis pendant 28 jours. Un génotypage par PCR a été utilisé pour distinguer recrudescence et réinfection. Les cas de réinfections ont été reclassifiés comme cas guéris.
RÉSULTATS Sur 176 enfants référés à la clinique d'étude, 126 satisfaisaient aux critères d'inclusions et ont été enrôlés. Aucun échec de traitement n'a été rapporté au début de l'étude. L'efficacité au jour 14 était de 98,2% (IC95%: 93,8–99,8). Sur 65 cas de parasitémie récurrente qui ont été analysés par PCR, 17 se sont avérés être de vraies recrudescences. L'efficacité au jour 14, ajustée par les résultats de PCR était de 84,5% (IC95%: 76,4–90,7). Tous les cas d'échec réel sont survenus dans les 8 derniers jours du suivi. Sur 110 enfants qui ont complété le suivi de 28 jours, 54 (49,1%) ont subi une nouvelle infection.
CONCLUSIONS L'efficacité de la combinaison était décevante. Le taux élevé des réinfections suggère un faible effet prophylactique. A Kailahun, une combinaison plus efficace reste nécessaire, quoique, peu d'alternatives soient disponibles en ce moment. L'efficacité de la combinaison artésunate plus amodiaquine devrait être suivie à Kailahun et dans d'autres régions de la Sierra Leone.

mots clés *Plasmodium falciparum*, thérapies de combinaison à l'artemisinine, artésunate, amodiaquine, efficacité in vivo, Sierra Leone

Baja eficacia de la combinación de artesunato con amodiaquina para el tratamiento de malaria por falciparum no complicada entre niños menores de 5 años en Kailahun, Sierra Leone

OBJETIVO: En el 2004, Sierra Leona adoptó el artesunato más amodiaquina como primera línea de tratamiento para malaria. Evaluamos la eficacia de esta combinación en Kailahun, en donde un estudio previo había demostrado una eficacia de la amodiaquina en monoterapia del 70.2%.
MÉTODOS: La metodología y la clasificación de los resultados cumplían con las directrices de la OMS. Los niños entre 6 y 59 meses, con malaria no complicada, fueron seguidos durante 28 días. Se utilizó el genotipaje mediante PCR para distinguir entre reinfección y recrudecimiento. Las reinfecciones fueron clasificadas como curados.
RESULTADOS: De 172 niños derivados a la clínica del estudio, 126 satisfacían los criterios de inclusión y entraron en el estudio. No se reportaron fallas terapéuticas tempranas. La eficacia a los 14 días fue del 98.2% (95% CI: 93.8–99.8). De 65 parasitemias recurrentes analizadas por PCR, 17 eran recrudescencias. La eficacia a día 28, ajustada por PCR, fue del 84.5% (95% CI: 76.4–90.7). Todas los fallos verdaderos ocurrieron durante los últimos 8 días de seguimiento. De 110 niños que terminaron los 28 días de seguimiento, 54 (49.1%) experimentaron una infección nueva.
CONCLUSIÓN: La eficacia de esta combinación fue decepcionante. La alta tasa de reinfección sugiere poco efecto profiláctico. En Kailahun se requiere una combinación más eficaz, aunque actualmente existen pocas alternativas disponibles. La eficacia de AS + AQ requiere monitorización tanto en Kailahun como en otras regiones de Sierra Leona.

palabras clave *Plasmodium falciparum*, terapia de combinación con artemisininas, artesunato, amodiaquina, eficacia in vivo, Sierra Leona