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Evidence basis for antimalarial policy change in Sierra Leone: five *in vivo* efficacy studies of chloroquine, sulphadoxine–pyrimethamine and amodiaquine

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Summary

OBJECTIVES To provide nationally relevant information on the antimalarial efficacy of chloroquine (CQ), sulphadoxine–pyrimethamine (SP) and amodiaquine (AQ) in Sierra Leone, with a view to updating antimalarial policy in the country.

METHODS Between October 2002 and May 2003, standard WHO methodology for *in vivo* efficacy assessment was used in five sites to study the therapeutic response of 6–59 months old uncomplicated *Plasmodium falciparum* malaria cases treated with CQ ($n = 247$), SP ($n = 353$) or AQ ($n = 434$). Follow-up was of 28 days, with polymerase chain reaction genotyping to distinguish late recrudescences from re-infections.

RESULTS Overall 85.3% of patients reached an analysable endpoint. CQ failure proportions were very high, ranging from 39.5% (95% CI: 25.0–55.6) in Kabala to 78.8% (65.3–88.9) in Kailahun. Early failures under CQ were frequent. SP efficacy was also disappointing, with failure from 23.2% (13.9–34.9) in Kabala to 46.1% (35.4–57.0) in Kailahun. AQ resistance was more moderate, ranging from 5.4% (1.8–12.1) in Makeni to 29.8% (20.3–40.8) in Kailahun, with almost no early failures. AQ also provided more rapid fever and parasite clearance.

CONCLUSION In a consensus meeting organized by the Ministry of Health and Sanitation, and based on these findings, artesunate (AS) + AQ and artemether–lumefantrine (Coartem™) were identified as the only options to rapidly replace CQ. The choice fell on AS + AQ because of expected high efficacy, lower cost in a blister presentation, and the absence of safety data on artemether–lumefantrine in pregnancy. Donor support is required to support this policy change. Throughout Africa, as SP resistance increases, these two regimens are probably the only options available while newer combinations are developed. Efficacy studies should focus on testing AQ and AS + AQ.

keywords *Plasmodium falciparum*, malaria, Sierra Leone, efficacy, chloroquine, sulphadoxine–pyrimethamine, amodiaquine, artesunate, policy

Introduction

In Sierra Leone, malaria is considered to be the top public health problem. Transmission (mostly of the lethal form, *Plasmodium falciparum*) is hyper- to holoendemic, with both entomological inoculation rate and parasite prevalence among the highest recorded anywhere (Barnish *et al.* 1993a). Malaria accounts for 48% of total outpatient

morbidity country-wide (although most of the diagnosis is presumptive), and data from the capital Freetown show 42% of inpatient paediatric deaths and 11% of maternal deaths are malaria related (Ministry of Health and Sanitation and Partners 2004). In southern Sierra Leone, 27% of community deaths were caused by malaria (Barnish *et al.* 1993b).

Despite this enormous disease burden, data on antimalarial efficacy were scarce. Armed conflict (1989–2000) severely damaged health infrastructures and hampered all malaria control efforts. In the mean time, chloroquine (CQ) and sulphadoxine–pyrimethamine (SP) remained respectively the first- and second-line regimens for uncomplicated malaria. The first post-war studies (2001) showed 8.3% failure of CQ in a small series carried out in the capital Freetown (Ministry of Health and Sanitation and Partners 2004), while in Matru, a town in the south-west (Figure 1), failure was 61.8% for CQ and 22.4% for SP (Bachy 2002). In order to update efficacy information on a national scale, and guide therapeutic policy change, we conducted simultaneous *in vivo* efficacy studies of CQ, SP and amodiaquine (AQ) at five different sites in Sierra Leone (Freetown, Kabala, Kailahun, Makeni and Matru). Data from a sixth site (Kenema) were rejected because of insufficient quality and are not reported here.

Materials and methods

Study sites and population

The studies were conducted in urban (Freetown), semi-urban (Makeni, Kabala) and rural (Kailahun, Matru) settings (Figure 1), selected because of their geographical spread, and due to the presence of a hospital-based outpatient department supported by Concern Worldwide (Freetown) or Médecins Sans Frontières (all other sites). In each site, malaria accounted for at least one-third of all consultations, admissions and inpatient deaths. Febrile children aged under 5 years were referred to the study from the routine hospital consultation, or nearby clinics. In Kailahun, Liberian refugees constituted a significant proportion of patients.

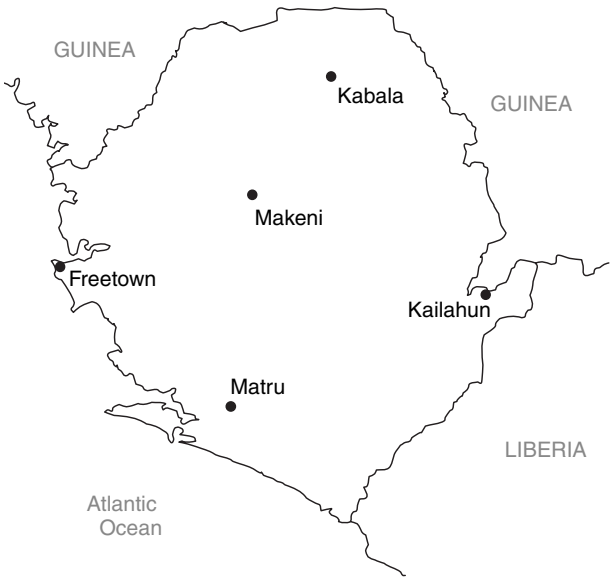

Figure 1 Location of study sites.

Design and procedures

The studies were approved by the Ethics Committee of the Sierra Leone Ministry of Health and Sanitation, and adhered to the latest WHO recommendations for antimalarial efficacy assessment in high transmission settings (WHO 2003a). Children 6–59 months old with uncomplicated malaria (defined as *P. falciparum* density 2000–200 000 asexual parasites per μl, no febrile co-infections as detected mainly by clinicians' presumptive diagnosis, no severe malnutrition, and no signs of severe malaria including severe anaemia defined as haemoglobin below 5 g/dl) were included (day 0) if their guardians consented in writing. Sites were instructed to include at least 50 (WHO 2003a) and, if possible, 97 (alpha 5%, precision 10% at worst) children per treatment arm. These sample sizes were increased by 10% to allow for defaulters and unclassifiable endpoints.

Upon inclusion, children received 3-day 25 mg/kg CQ (Nivaquine®, Rhône Poulenc), 1.25 mg/kg + 25 mg/kg SP stat (Fansidar®, Roche) or 3-day 30 mg/kg AQ (Camoquine®, Parke Davis), systematically allocated in a 1:1:1 ratio (except for Matru where only AQ was tested, as CQ and SP data were already available). All study doses were directly observed on days 0–2. Post-treatment visits occurred on days 3, 7, 14, 21, 28, or any other day in case of recurrent illness. At each visit, children were assessed clinically, axillary temperature was recorded, and thick and thin blood smears (stained with 3% Giemsa for 35 min) were inspected. Blind re-readings of each smear were performed on site, and a random sample of 60 to 100 per site independently reviewed at the Mbarara University of Science and Technology (Mbarara, Uganda). Haemoglobin was measured on days 0, 14 and 28 using a Lovibond® system (Assistant Co., Sondheim Rhon, Germany). Capillary blood spots were taken on 3 mm Whatmann filter paper on day 0 and the day of failure endpoint (see below), if this occurred after day 9.

Endpoint classification

Efficacy endpoints (failure or cure) were assigned based on the latest WHO classification (Table 1). In case of failure,

Table 1 Efficacy endpoint classification*

Endpoint	Criteria
Early treatment failure	Parasite density on day 2 > day 0 Parasite density on day 3 ≥ 25% of day 0 Fever in the presence of parasites on day 3 Severe malaria in the presence of parasites on days 1–3
Late clinical failure (days 4–28)	Fever in the presence of parasites on days 4–28 Severe malaria in the presence of parasites on days 4–28
Late parasitological failure (day 28)	Parasites without fever on day 28
Adequate clinical and parasitological response (day 28)	Follow-up completed without meeting any of the above criteria

* In a 14-day study, this same classification is used but endpoints at day 14 are considered.

children received a 7-day rescue course of quinine (except for Kailahun, where a combination of artesunate (AS) and SP was used). Children who, between days 4 and 28, had parasitaemia but no symptoms were monitored closely for clinical deterioration. No endpoint was assigned to children who were included outside of eligibility criteria, or who, after inclusion, vomited a study dose more than once, developed a serious febrile comorbidity, had a serious treatment-related adverse event, were infected with a non-falciparum species, received non-study antimalarials or antibiotics with antimalarial activity, or missed scheduled study visits.

In high-transmission settings, re-infections occur frequently and confound late clinical failure (LCF) and late parasitological failure (LPF) endpoints. So as to identify true failures (recrudescences) among these, polymerase chain reaction (PCR) genotyping analysis was performed at the Division of Infection and Immunity, University of Glasgow (Freetown, Kabala, Makeni sites) and at the Laboratoire de Parasitologie-Mycologie, Bichat Hospital, Paris (Kailahun, Matru sites) on *P. falciparum* DNA extracted from the day 0 and failure day filter spots. Procedures in these laboratories were mutually reviewed, and relied on well-described protocols (Ranford-Cart wright *et al.* 1997; Snounou *et al.* 1999) comparing the day 0 and failure day alleles of the merozoite surface proteins 1 and 2 (msp-1 and msp-2) gene loci (Standard Operating Procedures for the University of Glasgow methodology are freely available for download at http://www.gla.ac.uk/ibls/II/lrc/protocols.htm). LCF or LPF were confirmed as true recrudescences if msp-1 and msp-2 PCR amplification bands were similar on day 0 and the failure day. Conversely, cases in whom these bands were different were classified as re-infections. In case of simultaneous re-infection and recrudescence, the failure endpoint was retained.

Data analysis

Data were entered in duplicate on Microsoft Excel® and analysed per-protocol on SPSS® (SPSS Inc., Chicago, IL, USA). Patients without a clear efficacy endpoint were excluded from the analysis. In each arm, failures with missing PCR results were extrapolated as re-infections or recrudescences by applying the ratio observed among available results. Therefore, the total number of failures in each arm consisted of [early treatment failures (ETF) + LCF before or on day 9 + LCF and LPF confirmed as recrudescences by PCR + LCF and LPF with missing PCR result and extrapolated to be recrudescences]. Failure proportions at day 14 (without PCR adjustment) were also calculated. Baseline characteristics and failure proportions were compared within and among sites using chi-square (categorical variables) and ANOVA (continuous variables) tests.

Results

Study profile

Between October 2002 and May 2003, 2579 children were screened, of whom 1595 (61.8%) had a *P. falciparum* mono-infection, and 1034 (40.1%) met all inclusion criteria (Table 2). In Freetown, CQ allocation was increased (3:1:1 ratio) during the last 2 weeks of the study, so as to reach the minimum sample size (50) in that arm, considered critical for policy decisions. In Kabala, Kailahun and Makeni, CQ allocation was stopped early due to very poor efficacy. However, Kabala (CQ) failed to achieve the minimum sample size due to a number (nine) of parasitaemic, but not yet symptomatic, children receiving rescue treatment against protocol. In all arms but two, losses to follow-up were <10% (in Kailahun, a refugee camp was suddenly relocated). Other reasons for not assigning clear endpoints were: rescue treatment given to parasitaemic but asymptomatic children (34, 17 of whom in Kabala), febrile comorbidities (17), intake of non-study antimalarials (nine), repeated dose vomiting (four), non-falciparum infection (four), inclusion outside criteria (three), and one 56-month-old male case of severe pruritus on day 2 following AQ treatment in Makeni. In Freetown, one 8-month-old male (AQ arm) died in hospital on day 11

Table 2 Number (percentages) of patients included and analysable per study site and arm

Site/arm	Number included	No efficacy endpoint		Analysable at day	
		Lost to follow-up	Other reasons	14	28
Freetown	187				
CQ	71	4 (5.6)	8 (11.3)	61	59
SP	58	4 (6.9)	4 (6.9)	51	50
AQ	58	1 (1.7)	3 (5.2)	56	54
Kabala	227				
CQ	59	2 (3.4)	14 (23.7)	50	43
SP	85	6 (7.1)	10 (11.8)	80	69
AQ	83	12 (14.5)	5 (6.0)	74	66
Kailahun	259				
CQ	57	5 (8.8)	0 (0.0)	53	52
SP	101	10 (9.9)	2 (2.0)	94	89
AQ	101	14 (13.9)	3 (3.0)	92	84
Makeni	276				
CQ	60	4 (6.7)	6 (10.0)	56	50
SP	109	7 (6.4)	6 (5.5)	96	96
AQ	107	8 (7.5)	6 (5.5)	94	93
Matru	85				
AQ	85	3 (3.5)	5 (5.9)	78	77
Total	1034	80 (7.7)	72 (7.0)	935	882
Total CQ	247	15 (6.1)	28 (11.3)	220	204
Total SP	353	27 (7.6)	22 (6.2)	321	304
Total AQ	434	38 (8.8)	22 (5.1)	394	374

following a severe head wound. External quality control of slides yielded an endpoint-affecting discordance in <5% of cases for each study site.

Patient characteristics

Baseline age, axillary temperature, parasite density and middle-upper arm circumference differed significantly ($P < 0.001$) among sites (Table 3). Within sites, all arms were comparable except for Makeni, where CQ-treated children had significantly lower parasitaemia ($P = 0.007$) and were more anaemic ($P < 0.001$). Excluding Freetown (no haemoglobin results available), 88.2% (746/846) of children had any anaemia (haemoglobin <11 g/dl), and 33.5% (283/846) had moderate anaemia (haemoglobin <8 g/dl).

Treatment efficacy

The PCR-adjusted day 28 failure proportions (Table 4) were available for all arms except for Freetown CQ and SP, where >50% of failures had no blood spot collected. Overall, CQ performed very poorly, with up to three-quarters of patients failing treatment. ETF were very frequent, ranging from 16.3% (7/43) in Kabala to 34.6% (15/52) in Kailahun. SP failure was already near or above 25%, with ETF ranging from 6.3% (6/96) in Makeni to 18.0% (16/89) in Kailahun. AQ failure was relatively low everywhere except for Kailahun, and ETF were uniformly rare, with most failures occurring after day 14. AQ was superior ($P < 0.02$) to CQ everywhere, and significantly ($P < 0.03$) better than SP in Kailahun and Makeni (Freetown comparisons based on day 14 results).

On day 2, no significant differences in fever clearance were observed between CQ (range: 67.0% in Makeni to 82.9% in Freetown) and SP (range: 66.3% in Kailahun to 87.8% in Kabala), but in all AQ arms >95% of patients

Table 3 Patient characteristics at inclusion

Characteristic	Freetown ($n = 187$)	Kabala ($n = 227$)	Kailahun ($n = 259$)	Makeni ($n = 276$)	Matru ($n = 85$)
Age (months)	36	18	15	19	24
Median (IQR)	20–51	12–24	9–27	10–31	14–37
Parasite density (/μl)	18 999	26 402	17 394	28 159	28 615
Geometric mean (IQR)	7920–45 455	11 005–64 973	6800–41 666	11 603–70 543	12 500–65 425
Temperature (°C)	38.3	38.4	38.2	39.2	39.0
Median (IQR)	37.8–39.2	38.0–39.2	37.7–39.0	38.6–39.7	38.3–39.4
Gender	0.8	1.1	0.8	0.9	1.5
Ratio M/F	84/103	117/110	113/146	133/143	51/34
Haemoglobin (g/dl) *n* (%)					
Moderate anaemia (<8 g/dl)	Not reported	64 (28.2)	95 (36.7)	94 (34.2)	30 (35.3)
Mild anaemia (8–10.9 g/dl)		128 (56.4)	134 (51.7)	154 (56.0)	47 (55.3)
No anaemia (≥11 g/dl)		35 (15.4)	30 (11.6)	27 (9.8)	8 (9.4)
MUAC (mm)	149	146	147	143	151
Mean (IQR)	140–158	136–154	138–156	134–152	144–158

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

Table 4 Endpoints and failure proportions at day 14 (no PCR adjustment) and day 28 (PCR adjusted)

	Day 28 endpoints (before PCR)				Day 14 failure proportion (no PCR adjustment)			Day 28 failure proportion (PCR adjusted)		
Treatment/site	ETF	LCF	LPF	ACPR	*n*	%	95% CI	*n*	%	95% CI
CQ										
Freetown	15	10	15	19	37/61	60.7	47.3–72.9	40/59*	67.8	54.4–79.4
Kabala	7	18	6	12	30/50	60.0	45.2–73.6	17/43	39.5	25.0–55.6
Kailahun	18	21	9	4	39/53	73.6	59.7–84.7	41/52	78.8	65.3–88.9
Makeni	10	23	10	7	44/56	78.6	65.6–84.4	35/50	70.0	55.4–82.1
SP										
Freetown	4	4	6	36	9/51	17.6	8.4–30.9	14/50*	28.0	16.2–42.5
Kabala	9	5	6	49	14/80	17.5	9.9–27.6	16/69	23.2	13.9–34.9
Kailahun	16	23	7	43	29/94	30.9	21.7–41.2	41/89	46.1	35.4–57.0
Makeni	6	15	15	60	13/96	13.5	7.4–22.0	23/96	24.0	15.8–33.7
AQ										
Freetown	1	6	2	45	4/56	7.1	2.0–17.3	4/54	7.4	2.1–17.9
Kabala	2	9	14	41	8/74	10.8	4.8–20.2	12/66	18.2	9.8–29.6
Kailahun	1	30	19	34	9/92	9.8	4.6–17.8	25/84	29.8	20.3–40.7
Makeni	0	18	10	65	4/94	4.3	1.2–10.5	5/93	5.4	1.8–12.1
Matru	0	9	14	54	0/78	0.0	0.0–4.6	10/77	13.0	6.4–22.6

* No PCR adjustment. ACPR, adequate clinical and parasitological response.

were afebrile ($P < 0.02$ for all comparisons with AQ except Freetown AQ *vs.* SP). Less than half of CQ-treated patients were parasite-free on day 3 (range: 38.5% in Kabala to 47.3% in Makeni), and up to two-thirds of those receiving SP (range: 46.8% in Kabala to 70.4% in Freetown). AQ provided the most rapid parasite clearance (range: 56.8% in Kabala to 90.1% in Makeni; $P < 0.04$ for all comparisons with AQ except Kabala AQ *vs.* SP).

Discussion

These studies provide nationally relevant, synchronous and urgently needed data on antimalarial efficacy in Sierra Leone, a war-affected country with very low health indicators and a dramatically high malaria burden. Their main strength is the appropriately long patient follow-up (28 days), supported by PCR genotyping. Fourteen-day follow-up studies are easier to carry out, but seriously underestimate resistance, and should be avoided (Stepniewska *et al.* 2004).

Sampling in these studies was problematic, as none of the arms reached the ideal sample size (97), and one failed to reach the minimum (50). This was mainly due to insufficient human resources, notably doctors and skilled microscopists. In addition, the proportion of patients not reaching an endpoint was mostly higher than the 10% budgeted (WHO now recommends to plan for 20%). Our studies also relied on an imperfect method of treatment allocation, leaving open the possibility of prescriber bias. Block randomization with sealed envelopes would have been preferable, and made inter-arm comparisons more reliable. Losses to follow-up and protocol violations were, on the whole, acceptably low. However, a significant number of parasitaemic children received rescue treatment before day 28 although they could not yet be classified as LCF or LPF. The WHO methodology's requirement to withhold treatment in such cases was perceived as difficult to comply with by study clinicians, notably in Kabala for CQ-treated children. Excluding these 'probable failures' from the analysis underestimates resistance, and explains why in Kabala CQ failure seemed lower than elsewhere.

Despite these drawbacks, clear trends emerge from these studies. Antimalarial resistance has reached crisis proportions in Sierra Leone. CQ, the first-line regimen, fails to cure most children who receive it, and, for many, does not even provide temporary improvement. SP resistance is also advanced, although the drug has not been used as first-line. SP-resistant strains are rapidly spreading throughout Africa and are favoured by high transmission (Talisuna *et al.* 2002, 2004). AQ remains acceptably efficacious in Sierra Leone, and provides fast fever and parasite clearance. AQ resistance seems more stable over time (The East African Network for Monitoring Antimalarial Treatment (EANMAT) 2003). Efficacy of all three drugs was poorest in Kailahun: possible reasons include imperfect tablet dispensation, younger age and thus lower immunity of included children, and introduction of highly resistant strains from neighbouring Liberia, where studies have

shown very high CQ and SP (Checchi *et al.* 2002a) and moderate AQ (Checchi *et al.* 2002b) resistance. Site-specific patterns of health service utilization (which we did not inquire about) might also explain geographical differences in efficacy. Where home remedies and drug sellers are often the first curative choices, public structures might see proportionally more resistant infections (i.e. cases that cannot be managed at home), thus skewing the profile of children referred to the study.

Antimalarial resistance of *P. falciparum* is constantly evolving. Failure to rapidly detect a decline in drug efficacy (in Sierra Leone's case, a consequence of wartime neglect) leads to greater morbidity and mortality (Trape 2001; Zucker *et al.* 2003), and greatly complicates malaria control. Resistant cases are at higher risk of severe malaria (Olumese *et al.* 2002) and anaemia (Bjorkman 2002), are more infectious due to persistent parasitaemia and higher gametocyte carriage (Bousema *et al.* 2003), and their diagnosis and management are more difficult and expensive. Our results highlight the urgent need to move away from CQ, and monotherapies in general, in Sierra Leone.

To address our findings, a consensus meeting was called by the Ministry of Health and Sanitation in March 2004, with participation from central government, district medical authorities, WHO, donors, and the NGO community. There was wide consensus that CQ use should be discontinued, and that combination therapy should replace it so as to improve cure rates and protect partner drugs against further spread of resistant strains (Nosten & Brasseur 2002). The therapeutic lifespan of any SP-based combination would probably be short, necessitating extensive efficacy monitoring and an additional policy change in the near future. In accordance with current WHO recommendations (WHO 2003b), the choice was thus narrowed down to AS + AQ and artemether–lumefantrine (Coartem^{TM}), and the former was selected. Both combinations are well tolerated (Bakshi *et al.* 2000; Adjuik *et al.* 2004). Artemether–lumefantrine is highly efficacious (van Vugt *et al.* 1999; Epicentre, unpublished data), but its safety in pregnancy has not yet been established, and it currently costs more than AS + AQ (2.4 USD *vs.* 1.5 USD per adult blister pack). Given the moderate AQ failure proportions observed in Sierra Leone, AS + AQ efficacy is expected to be high, as shown elsewhere (Adjuik *et al.* 2002), although it may need regular monitoring given the somewhat alarming results from Kailahun (Rwagacondo *et al.* 2004). Progressive phasing in of the combination should be completed in 2006. In addition, SP monotherapy was retained for intermittent preventive treatment (IPT) in pregnancy given the absence of safety data on other potential IPT regimens.

Conclusion

Today, Sierra Leone faces a daunting task of rolling back malaria. The decision to replace CQ with an efficacious artemisinin-based combination is a crucial step in the right direction. This policy merits a sustained commitment from international donors to support country-wide deployment of AS + AQ, and ensure the combination is financially affordable for all citizens. Elsewhere in Africa, the assumptions today, even where data are missing, should be that CQ no longer works, and that SP efficacy is waning. Efficacy studies should instead focus on selecting efficacious alternatives. However, the choice may be increasingly limited to AS + AQ and artemether–lumefantrine, at least while newer combinations such as dehydroartemisinin–piperaquine and AS–chlorproguanil–dapsone complete regulatory trials (Olliaro & Taylor 2003). For pregnancy, there may not be a choice at all. As there are no reports yet of *in vivo* resistance to artemether–lumefantrine, reproducing efficacy tests of this combination may have limited benefit. By contrast, studies of AQ and AS + AQ seem presently most useful to guide therapeutic policy change.

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