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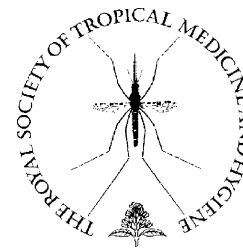
Efficacy of chloroquine, sulfadoxine—pyrimethamine and amodiaquine for treatment of uncomplicated *Plasmodium falciparum* malaria among children under five in Bongor and Koumra, Chad

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Summary   We report two 28-day in-vivo antimalarial efficacy studies carried out in the urban centres of Bongor and Koumra, southern Chad. We assess chloroquine (CQ), sulfadoxine—pyrimethamine (SP) and amodiaquine (AQ) to treat *Plasmodium falciparum* uncomplicated malaria. Methods and outcome classification complied with latest WHO guidelines. Out of the 301 and 318 children aged 6—59 months included in Bongor and Koumra, respectively, 246 (81.7%) and 257 (80.8%) were eligible for analysis. In Bongor and Koumra, the 28-day PCR-adjusted failure rates for CQ were 23.7% (95% CI 14.7—34.8%) and 32.9% (95% CI 22.1—45.1%), respectively, and those for SP were 16.3% (95% CI 9.4—25.5%) and 4.3% (95% CI 1.2—10.5%). AQ failure rates were 6.4% (95% CI 2.1—14.3%) and 2.2% (95% CI 0.3—7.6%). The current use of CQ in Bongor and Koumra is questionable, and a more efficacious treatment is needed. Considering the reduced efficacy of SP in Bongor, AQ seems to be the best option for the time being. Following WHO recommendations that prioritize the use of artemisinin-based combinations, artesunate plus amodiaquine could be a potential

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1. Introduction

Resistance of *Plasmodium falciparum* to conventional monotherapies is a major constraint for malaria control. Resistance to chloroquine (CQ) and sulfadoxine—pyrimethamine (SP) has reached high levels in many sub-Saharan countries from Eastern Africa (Bayoumi et al., 1989; Rønn et al., 1996; Talisuna et al., 2002; Terlouw et al., 2003) to Western Africa (Checchi et al., 2002; Ekanem et al., 1990; Sowunmi et al., 2001). This phenomenon has led to an increase in disease-specific morbidity and mortality (Marsh, 1998; Trape, 2001), and the lack of efficacious and affordable treatments has severely constrained the public health response in many countries.

The use of artemisinin-based combination therapies (ACTs) is currently seen as the best option for the treatment of malaria (Nosten and Brasseur, 2002; White and Olliaro, 1996; White et al., 1999) because of their high efficacy and their potential to reduce the risk for resistance. WHO recommends that countries experiencing resistance to monotherapies shift to combination therapies, preferably to those containing an artemisinin derivative (WHO, 2001).

In Chad, malaria is a major public health problem, representing 19.7% of the total cases at outpatient services (Ministry of Health, unpublished data). Little information on the efficacy of CQ, the first-line treatment for uncomplicated malaria, is available. According to the national health information system, in 2000 CQ resistance was 6.2% (Ministry of Health, unpublished data). This figure, collected through routine reporting activities, might not accurately reflect the real situation. In addition, no information on SP efficacy is available. In order to fill this gap, two studies were carried out by Médecins sans Frontières, Epicentre and the Chadian Ministry of Health to evaluate the efficacy of the two recommended antimalarial drugs, CQ and SP, and that of amodiaquine (AQ). The latter was considered a potential alternative because of its low cost and its infrequent use. The studies were carried out in 2002 in Bongor and in 2003 in Koumra, both towns located in southern Chad.

2. Materials and methods

2.1. Study sites

Bongor (population 51 000) is the administrative centre of Mayo-Kebbi prefecture, located 240 km south of N'Djamena along the Logone River, in proximity with the border with Cameroon (Figure 1). The 128-bed hospital, located in the centre of the city, is the only referral health care facility for the district. Several urban health centres (UHCs) serve as first-line heath care facilities. Malaria accounts for approximately one-fifth of all consultations. Two UHCs, located within walking distance from the hospital, participated in the study.

Koumra, with an estimated population of 43 000, is located in the southern-central prefecture of Moyen-Chari, on the border of the Central African Republic. There are two hospitals of 124 and 206 beds in the town. The study was hosted in the UHC of Koumra North.

Plasmodium falciparum was reported as responsible for the majority of malaria cases in both towns. Parasite transmission is perennial, with a marked increase during the rainy season between July and October.

2.2. Study design

Both studies followed WHO guidelines for assessment of therapeutic efficacy of antimalarial drugs for uncomplicated falciparum malaria in areas with intense transmission (WHO, 1996, 2002). Efficacy results were analysed according to

Figure 1 Location of Bongor and Koumra, Chad.

the latest WHO guidelines (WHO, 2003). Children aged 6 to 59 months with suspicion of malaria (axillary temperature ≥37.5 °C) were referred to the study clinic. If eligible for inclusion (discussed below), a written informed consent was sought. The child received one of the study drugs under direct observation and was followed-up for 28 days. Drug assignment was done consecutively in batches. The first batch was CQ, followed by SP and then AQ.

2.3. Sample sizes

As no accurate information on efficacy of the study drugs was available in either study site, sample size estimates were based on 50% failure proportion for each drug. Applying a level of significance of 95% and a precision of 10%, 96 children per treatment arm were required. Allowing for a 10% loss to follow-up, the final sample size was thus raised to 106 children per treatment arm, i.e. 318 in total in each site.

2.4. Enrolment and follow-up of study patients

A thick and thin blood film was obtained from children referred with suspected malaria. Haemoglobin was measured and a capillary blood sample was collected on filter paper for PCR genotyping. A physician examined the child's clinical condition and assessed whether all criteria for inclusion had been met. Children were eligible for the study if they had all of the following: (1) were aged 6—59 months; (2) had fever (axillary temperature ≥37.5 °C); (3) had *Plasmodium falciparum* mono-infection with asexual count 2000—100 000/µl; and (4) were likely to complete the 28-day follow-up (i.e. living within 10 km or 2 h walk from the clinic). Children were not eligible if they had any of the following: (1) signs of severe illness or severe malaria (WHO, 1996); (2) concomitant severe infectious disease; (3) severe malnutrition (MUAC <110 mm and/or presence of bilateral oedema); (4) taken a complete antimalarial treatment during the previous 7 days; or (5) history of allergy to the drug to be administered. Written informed consent was obtained from the parent or the guardian of each child enrolled.

After enrolment (day 0), the children were requested to come to the clinic on a fixed schedule at day 1, 2, 3, 7, 14, 21 and 28 for a clinical examination. Study drug intake was completed at days 1 and 2. Starting from day 2, along the fixed schedule, and any time it was clinically warranted, a parasitological assessment was also performed.

2.5. Treatments

Enrolled children received: (1) chloroquine (100 mg base tablets, Rhône Poulenc Rorer, France), 10 mg/kg at day 0, 10 mg/kg at day 1 and 5 mg/kg at day 2; or (2) sulfadoxine—pyrimethamine (500 mg sulfadoxine + 25 mg pyrimethamine tablets, Fansidar®, Roche, France), 1.25 mg/kg bodyweight pyrimethamine at day 0; or (3) amodiaquine (in Bongor 200 mg base tablets, Camoquin Park Davis, Senegal; in Koumra 200 mg base tablets, Pfizer, West Africa), 10 mg/kg/day at day 0, day 1 and day 2. Dosages were expressed as fraction of tablets and adapted to simplify prescription at the outpatient department.

The child received paracetamol if clinical conditions warranted. In case of any therapeutic failure (see below), the child received an oral rescue treatment consisting of quinine base 8 mg/kg, every 8 h for 7 days. If the oral intake was not possible, the child was referred to the hospital.

2.6. Therapeutic outcomes

Patients were classified as (1) early treatment failure (ETF), (2) late clinical failure (LCF), (3) late parasitological failure (LPF), or (4) adequate clinical and parasitological response (ACPR), as per WHO guidelines (WHO, 2003).

Children were withdrawn from the study at any time during the follow-up period if they met one or more of the following criteria: (1) failure to take any study drug dose; (2) development of an allergic reaction to the study drug or a side effect severe enough that alternative treatment needed to be prescribed; (3) detection of a non-falciparum mixed or mono-infection; (4) occurrence of a severe infectious disease; (5) self-medication with any antimalarials during follow-up; or (6) withdrawal of consent by the parent or the guardian. Children were classified as lost to follow-up if they did not attend the day 28 visit (one day of delay was accepted).

2.7. Laboratory procedures

Microscope examination was done under 100× oil immersion magnification, according to WHO guidelines (WHO, 1996). In brief, asexual parasite density/µl was computed by counting the number of trophozoites against 200 white blood cells (WBCs) and assuming a standard WBC density of 8000/µl. Species determination was done on the thin blood film. A thick blood film was considered negative when no asexual parasite was found in 100 fields. Two laboratory technicians double-read all slides systematically. In the event of discordant results, such as difference in species diagnosis, or difference in parasite density >50%, a further reading was performed by a third laboratory technician. Independent laboratories performed the external quality control of 95 randomly chosen samples from Bongor and 98 from Koumra. For all slides controlled by the independent laboratory, only one discordant result compared with the study laboratory would have yielded a different outcome classification. Haemoglobin was measured using the Lovibond undiluted colorimetric technique (Lovibond, Assistant Co. Sondheim Rhon, Germany).

2.8. PCR genotyping technique

Samples for PCR analysis were collected on Whatman No. 3 filter paper, air-dried and stored in separated sealed bags in dry and dark conditions at room temperature. Samples from Bongor were analysed for *msp-1* and *msp-2* markers at the Prince Leopold Institute of Tropical Medicine, Antwerp (Belgium), using a previously described method (Ranford-Cartwright et al., 1993). Samples from Koumra were analysed for *glurp* first, and then *msp-1* and *msp-2* markers, if needed, at the Epicentre laboratory at the Mbarara University of Science and Technology, Mbarara (Uganda),

according to a published nested PCR method (Snounou, 2002).

Pre- and post-treatment samples were compared and possible outcomes were: (1) recrudescence, if the alleles of the pre- and post-treatment samples were the same for *msp-1* and *msp-2* (Bongor) or for *glurp, msp-1* and *msp-2* (Koumra); (2) re-infection, if the alleles of the pre- and post-treatment samples were distinct; (3) mixed recrudescence and re-infection, if similar alleles were found in the pre- and post-treatment samples for all the markers as mentioned above, but with additional distinct alleles identified; (4) indeterminate, if at least one marker in either the pre- or the post-treatment sample did not allow a definitive conclusion; or (5) no DNA isolated, if one or both the pre- and post-treatment samples could not be amplified.

2.9. Data entry and analysis

Data from Bongor were entered into a locked Excel spreadsheet and 10% of entries were verified. Data in Koumra were double-entered and validated using EpiData v2.1b (The Epidata Association, Odense, Denmark). Both datasets were analysed using Stata v8.0 (Stata Corp., College Station, TX, USA). Baseline characteristics of patients in each group were compared using a χ^2 test for dichotomous variables, ANOVA for continuous variables approaching a normal distribution, and the Kruskal-Wallis test for non-normally distributed or discrete numerical variables. Wilcoxon matched pair tests were used to compare day 0 and day 28 haemoglobins. Failure proportions at day 28 were calculated as the total number of failures (ETF + LCF + LPF) over the total number of analysable outcomes, and expressed as a percentage with associated 95% confidence intervals. PCR analysis results were used to adjust failure proportions at day 28. Only treatment failures, proved to be recrudescence or mixed recrudescence and re-infection, were classified as true failures. Patients for which the PCR result was inconclusive (indeterminate or no DNA isolated) or reported as a pure re-infection, patients lost to follow-up, and secondary exclusions were all excluded from the analysis.

3. Results

3.1. Bongor

Between August and December 2002, a total of 736 children aged 6 to 59 months with suspicion of malaria were referred to the study clinic (Figure 2). Of these, 301 met all required inclusion and exclusion criteria; 106 were assigned each to the CQ and SP arms, 89 to the AQ arm. After exclusions during follow-up, the number of patients analysable at day 28 was 76, 92 and 78 in each of these arms, respectively. Children were similar in their baseline characteristics with the exception of axillary temperature and haemoglobin level (Table 1). Notably, children in the CQ group were more anaemic than those in the SP and AQ groups ($P = 0.002$). Parasitaemia was generally high in the three groups: on average 68.8% of children (207/301) had a density above 10 000 parasites/μl.

Clinical failures (ETF + LCF) at day 14 were 13/98 (13.3%, 95% CI 7.3—21.6%) in the group treated with CQ, 16/100 (16.0%, 95% CI 9.4—24.7%) in the SP group, and 2/80 (2.5%, 95% CI 0.3—8.7%) in the AQ group. Parasitological and clinical outcomes at day 28 are shown in Table 2. Total failures at
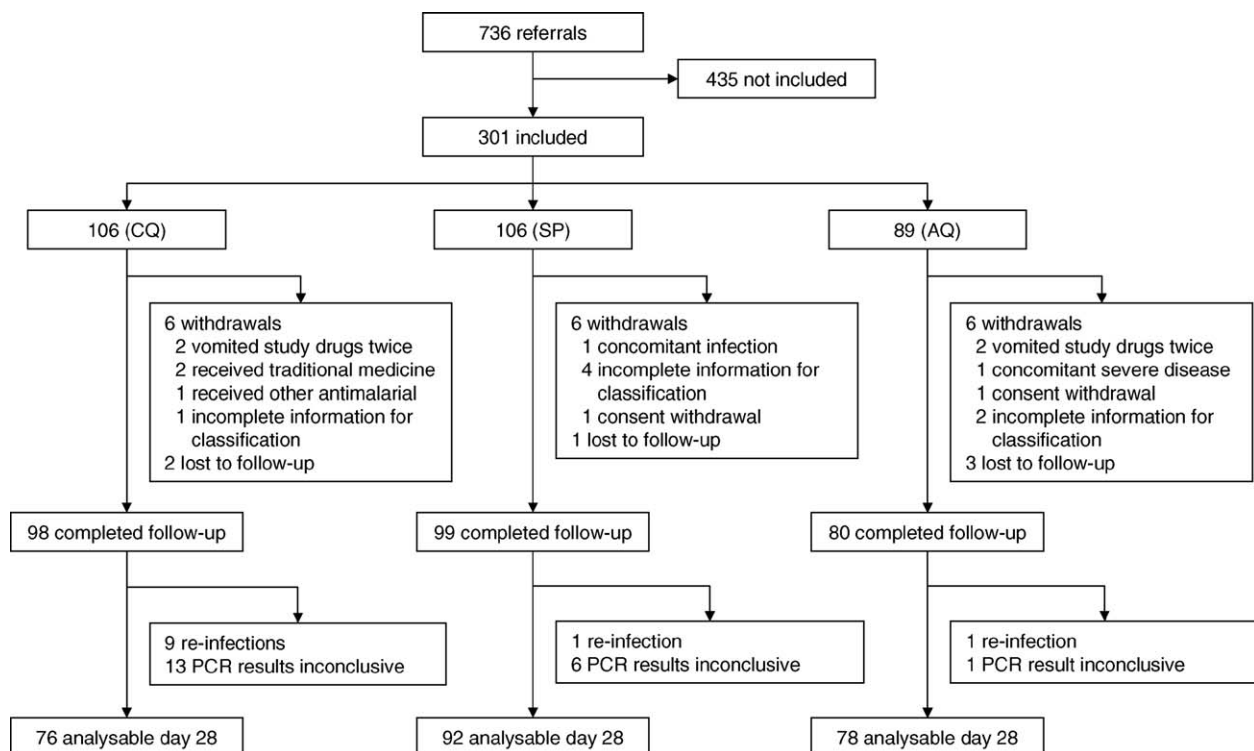

Figure 2 Study enrolment details for 736 children with clinically suspected malaria, Bongor, Chad, 2002.

Table 1 Baseline characteristics at enrolment of study children in Bongor (2002) and Koumra (2003), Chad

Characteristic	Bongor				Koumra			
	CQ (n = 106)	SP (n = 106)	AQ (n = 89)	P	CQ (n = 106)	SP (n = 106)	AQ (n = 106)	P
Age (months), Median (IQR)[a]	30 (18—38)	31 (24—48)	32 (20—40)	0.6	24 (14—36)	24 (15—40)	24 (17—36)	1.0
Gender ratio (M/F)	1.3 (59/47)	0.9 (51/55)	1.2 (48/41)	0.5	1.2 (57/49)	1.1 (56/50)	1.2 (57/49)	1.0
Axillary temperature (°C), Median (IQR)[a]	38.9 (38.0—39.8)	38.5 (37.5—39.5)	38.5 (37.5—39.5)	0.02	38.5 (35.5—39.9)	38.9 (38—39.8)	38.5 (37.5—39.5)	0.08
Parasitaemia/μl, Geometric mean (IQR)[a]	18 245 (8280—44 426)	17 571 (8112—38 880)	15 347 (6320—34 978)	0.5	17 132 (8082—38 701)	19 521 (10 852—41 558)	21 798 (8121—62 306)	0.3
Parasite density >10 000/μl, n (%)	75 (70.8)	73 (68.9)	59 (66.3)	0.8	74 (69.8)	81 (76.4)	74 (69.8)	0.5
Haemoglobin level, median (IQR)[a]	10.7 (8.7—11.7)	11.2 (10.2—12.2)	10.7 (10.2—11.7)	0.002	9.7 (7.3—10.7)	8.7 (7.3—10.7)	8.0 (6.7—9.7)	<0.001

[a] 25—75% interquartile range.

Table 2 Day-28 PCR adjusted therapeutic response of study children to chloroquine, sulfadoxine—pyrimethamine and amodiaquine in Bongor (2002) and Koumra (2003), Chad

Outcome	Chloroquine			Sulfadoxine—pyrimethamine			Amodiaquine		
	n	%	95% CI	n	%	95% CI	n	%	95% CI
Bongor									
Early treatment failure	7	9.2	3.8—18.1	11	12.0	6.1—20.4	2	2.6	0.3—9.0
Late clinical failure	6	7.9	3.0—16.4	4	4.3	1.2—10.8	1	1.3	0.0—6.9
Late parasitological failure	5	6.6	2.2—14.7	0	0.0	0.0—3.9[a]	2	2.6	0.3—9.0
Total failure	18	23.7	14.7—34.8	15	16.3	9.4—25.5	5	6.4	2.1—14.3
Adequate clinical and parasitological response	58	76.3	65.2—85.3	77	83.7	74.5—90.6	73	93.6	85.7—97.9
Total	76	100.0		92	100.0		78	100.0	
Koumra									
Early treatment failure	8	11.4	5.1—21.3	4	4.3	1.2—10.5	0	0.0	0.0—3.9[a]
Late clinical failure	15	21.4	12.5—32.9	0	0.0	0.0—3.8[a]	1	1.1	0.03—5.8
Late parasitological failure	0	0.0	0.0—5.1[a]	0	0.0	0.0—3.8[a]	1	1.1	0.03—5.8
Total failure	23	32.9	22.1—45.1	4	4.3	1.2—10.5	2	2.2	0.3—7.6
Adequate clinical and parasitological response	47	67.1	54.9—77.9	90	95.7	89.5—98.8	91	97.8	92.4—99.7
Total	70	100.0		94	100.0		93	100.0	

[a] One-sided 97.5% confidence interval.

day 28, after PCR adjustment, accounted for 18/76 (23.7%, 95% CI 14.7−34.8%) in the group treated with CQ, 15/92 (16.3%, 95% CI 9.4−25.5%) in the SP group, and 5/78 (6.4%, 95% CI 2.1−14.3%) in the AQ group. Early treatment failures were frequent in the CQ and SP groups: 7/76 (9.2%, 95% CI 3.8−18.1%) and 11/92 (12.0%, 95% CI 6.1−20.4%), respectively.

Among children who completed day 28 follow-up, and regardless of the treatment outcome, there was a significant improvement in haemoglobin level for all treatment groups (P < 0.001, data not shown).

3.2. Koumra

Between August and November 2003, 898 children 6 to 59 months of age with suspicion of malaria were screened, and 318 (106 for each treatment group), fitting all criteria, were included (Figure 3). After exclusions during follow-up, the number of patients analysable at day 28 was 70, 94 and 93 in the CQ, SP and AQ groups, respectively. As in Bongor, children were similar for baseline characteristics at inclusion apart for haemoglobin (Table 1). By contrast to Bongor, children in the CQ group (the first to be included) were significantly less anaemic than those in the SP and the AQ groups (P < 0.001). Parasitaemia was also generally high: 72.0% of children (229/318) had a density above 10 000 parasites/μl, but no difference between treatment groups for this and any other baseline characteristics was found.

Clinical failures (ETF + LCF) at day 14 were 21/98 (21.4%, 95% CI 13.8−30.9%) in the group treated with CQ, 4/100 (4.0%, 95% CI 1.1−9.9%) in the SP group, and 2/104 (1.9%, 95% CI 0.2−6.8%) in the AQ group. Parasitological and clinical outcomes at day 28 are shown in Table 2. Total failures at

day 28 after PCR adjustment, were 23/70, (32.9%, 95% CI 22.1−45.1%) in the group treated with CQ and 4/94 (4.3%, 95% CI 1.2−10.5%) and 2/91 (2.2%, 95% CI 0.3−7.6%) in the SP and AQ groups, respectively.

Early treatment failures were high in the CQ group: 8/70 (11.4%, 95% CI 5.1−21.3%). In the SP group, all four failures occurred in the first 3 days of follow-up. No early treatment failures were reported among children treated with AQ. The two failures in this group were one late clinical failure, which occurred at day 7, and one late parasitological failure at day 28.

As in Bongor, among children who completed day 28 follow-up, and regardless of the treatment outcome, there was a significant improvement in haemoglobin level for all treatment groups (P < 0.001, data not shown).

4. Discussion

The results of these two studies provide estimates of the in-vivo antimalarial efficacy in Chad, where little and imprecise information about CQ, and no information about SP and AQ, was available. They may also provide additional important information for decisions on the appropriate treatment strategy for this region, thus contributing to a clearer overall picture of antimalarial drug resistance in Africa.

Losses to follow-up and withdrawals from the studies were acceptably low, and the external quality control showed reliable study laboratory results. Moreover, the 28-day follow-up, combined with PCR analysis, provided more realistic estimates of efficacy than traditional 14-day studies (Stepniewska et al., 2004). The major limitation of our studies was the method of treatment allocation, which was not randomized. This could explain the unequal level of
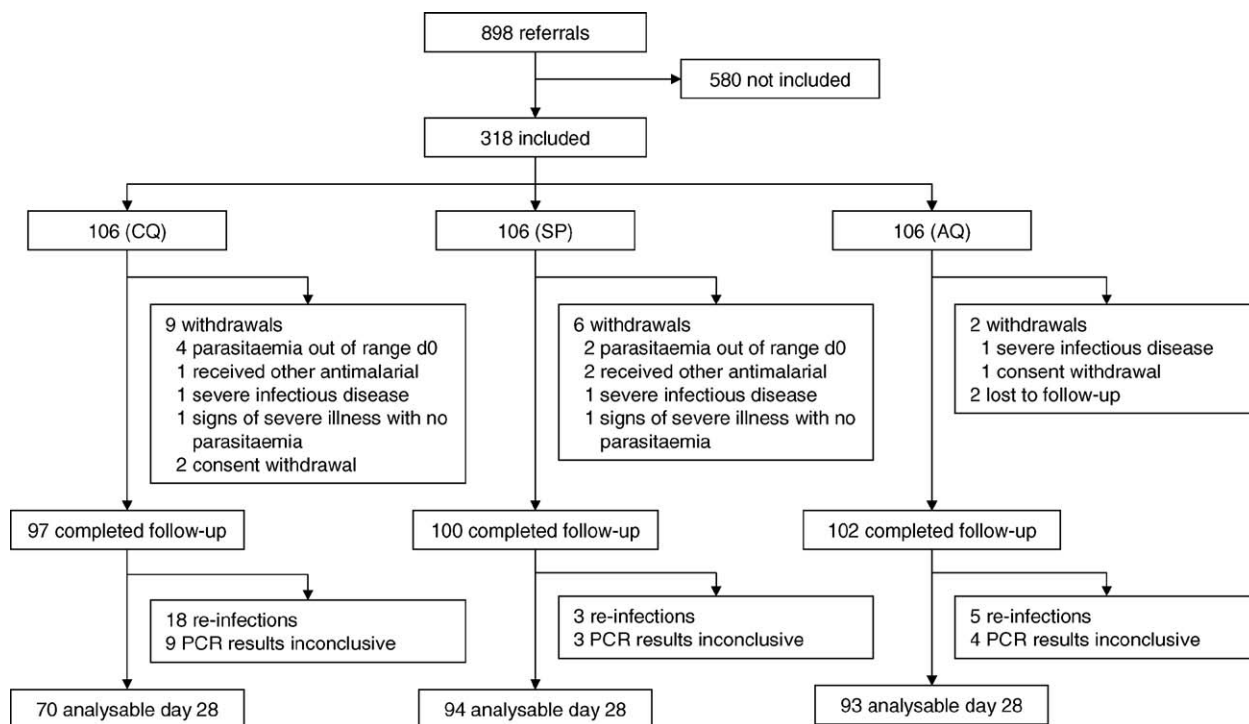

Figure 3   Study enrolment details for 898 children with clinically suspected malaria, Koumra, Chad, 2003.

haemoglobin at inclusion in the three groups and did not allow for a rigorous comparison of the results.

Despite this limitation, some clear issues emerged from these studies. The in-vivo efficacy of CQ, the first-line anti-malarial treatment in Chad, was clearly low. In the 28-day follow-up, CQ failed to cure almost one-quarter of children in Bongor and one-third in Koumra, with an alarming proportion of early failures in both sites. Although CQ failure proportions seemed higher than the figures reported by the routine Chadian surveillance system, these results were not surprising. A lower efficacy (58.5%) was already reported in neighbouring Nigeria (Sowunmi et al., 2001) and was extensively reported in many other sub-Saharan countries (Bayoumi et al., 1989; Checchi et al., 2002; Talisuna et al., 2002).

In both Bongor and Koumra, day-14 clinical failures to CQ reached — or were about to reach — the 15% threshold, when, as indicated by WHO, a change to a more efficacious treatment is necessary (WHO, 2003). Performance worsened with a 28-day follow-up. Such low efficacy is likely to lead to increased malaria-related morbidity and mortality (Trape, 2001), and the use of CQ is therefore to be discouraged.

The efficacy of SP in Bongor was relatively low, while a better result was reported in Koumra. There is no clear reason for this relatively big difference between the two towns. Bongor, although similar in size to Koumra, is closer to the capital and commercially more active; it is possible that the more developed private health sector in Bongor introduced the use of SP earlier and more consistently, contributing to a faster selection of resistant parasites.

Failures to this drug had, however, a common pattern in the two sites. While in the CQ and AQ groups the failures were more evenly distributed throughout the 28-day follow-up, all four treatment failures to SP in Koumra and almost all those in Bongor (11 failures out of 15) occurred during the first 3 days after the first drug intake. Resistance to SP develops rapidly (Bloland, 2001), and similar studies recently reported in the near Sudan (Stivanello et al., 2004) and Uganda (Checchi et al., 2004) already showed unacceptably high levels of resistance. Considering its relatively low efficacy in Bongor and the rapid development of resistance, SP does not seem an appropriate alternative to CQ as first-line treatment in Chad.

AQ appeared the best option among the three regimens tested. The efficacy of this treatment was high both in Bongor and Koumra. Our results are consistent with other studies from neighbouring countries, e.g. Nigeria (Sowunmi et al., 2001) and Cameroon (Ringwald et al., 2000), where the efficacy of AQ was more than 95%. Moreover, it seems that its efficacy is more stable than that of SP over time (EANMAT, 2003), although cross-resistance with CQ has been shown in several settings. This means that the risk of increasing resistance to AQ should always be considered, particularly in areas where resistance to CQ is high (Rwagacondo et al., 2004).

In recent years, a new strategy to hold parasite resistance in check has been the use of appropriate combination therapies, which reduces the probability of selection of resistant parasite strains by a mechanism of mutual protection (Nosten and Brasseur, 2002; White and Olliaro, 1996). In this perspective, WHO currently recommends that African countries prioritize artemisinin-based combinations (WHO, 2001). Suitable combinations for Bongor and Koumra would be artesunate plus amodiaquine (AS + AQ) or artemether plus lumefantrine (Coartem®). The choice of a new first-line treatment is determined by different factors, such as drug availability, acceptability, cost and patient compliance. Coartem has the advantage of being the only fixed combination still available on the market, and recently it has been shown to be highly efficacious in African settings (Mutabingwa et al., 2005; Piola et al., 2005). However, when compared with Coartem, the lower cost and the lower drug intake (once daily versus twice daily for Coartem), make AS + AQ a more suitable choice for first-line treatment in areas, where, like Bongor and Koumra, the efficacy of monotherapy AQ is still high. Although (unlike Coartem) the AS + AQ is not a fixed-combination, the current co-blister pack presentation should favour patient compliance.

All ACTs are more expensive than conventional monotherapies such as CQ and SP. International mechanisms should be put in place to support the necessary funding for the additional cost. The advantages of combination therapies in malaria are widely recognized. However, in order to maximize the benefits, the change to ACTs needs to be thoroughly prepared, implemented and monitored.

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