

Chloroquine, sulfadoxine–pyrimethamine and amodiaquine efficacy for the treatment of uncomplicated *Plasmodium falciparum* malaria in Upper Nile, South Sudan

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Abstract

The current first-line and second-line drugs for *Plasmodium falciparum* malaria in South Sudan, chloroquine and sulfadoxine–pyrimethamine (SP), were evaluated and compared with amodiaquine, in an MSF-Holland-run clinic in eastern Upper Nile, South Sudan from June to December 2001. Patients with uncomplicated malaria and fever were stratified by age group and randomly allocated to one of 3 treatment regimes. A total of 342 patients was admitted and followed for 14 d after treatment. The drop-out rate was 10.2%. Of those who completed the study, 104 were treated with chloroquine (25 mg/kg, 3 d), 102 with SP (25 mg/kg sulfadoxine and 1.25 mg/kg pyrimethamine, single dose) and 101 with amodiaquine (25 mg/kg, 3 d). Adequate clinical response was observed in 88.5% of patients treated with chloroquine, 100% of patients treated with SP and 94.1% of patients treated with amodiaquine. In children aged < 5 years, the success rate was lower: 83.3% for chloroquine and 93.0% for amodiaquine. In adults no treatment failures were found, but children aged 5–15 years showed intermediate levels. In addition, we determined the initial genotypes of *dhfr* and *dhps* of 44 isolates from the SP-treated group and > 80% were found to be wild type for *dhfr* and 100% for *dhps*. Two percent of isolates had a single mutation and 16% had double mutations of *dhfr*. These data are in full agreement with the clinical effectiveness of SP. A change in malaria treatment protocols for South Sudan is recommended.

Keywords: malaria, *Plasmodium falciparum*, chemotherapy, resistance, chloroquine, sulfadoxine–pyrimethamine, amodiaquine, genotypes, South Sudan

Introduction

Background and justification

In the Sudan, the long-lasting war between the government in the North and rebel groups in the South has left the southern part of the country isolated, with poor infrastructure, system of governance, trade, food production, education and health services. Health services in South Sudan rely almost fully on support from foreign agencies. Continuing violent conflicts among factions in the area itself have created an insecure, unstable environment. This is a serious constraint for international aid agencies working in the area, resulting in a patchy and often temporary presence of relief projects. Malaria is a leading cause of morbidity in South Sudan (WHO/CDS/RBM, 2000). However, under the present conditions, few health providers recognize malaria as a life-threatening disease that needs specific attention (Allan, 2001). Among the various severe diseases prevalent, such as visceral leishmaniasis, sleeping sickness, tuberculosis (TB) and epidemic-prone meningitis or diarrhoeal diseases, malaria might be overlooked. Mortality and morbidity rates from malaria in southern Sudan have risen during the lasting conflict, because of: (i) limited access to health facilities and drugs; (ii) insecurity, driving people to areas of higher endemicity or mosquito-infested marshland; (iii) no means of protection from mosquitoes (secure houses, clothes, mosquito nets); and (iv) decreased immunity due to chronic diseases and malnutrition.

Plasmodium falciparum resistance to antimalarials in South Sudan and surrounding areas

In 1994–95, several health agencies surveyed chloroquine resistance in southern Sudan. They found a chloroquine failure rate of 5–10% in Upper Nile (Duar, Waat and Akobo) and Bahr El Ghazal (Tambura), using various study protocols as described by

Médecins du Monde (1996) and in unpublished reports. A follow-up study in Duar revealed 27% chloroquine failure in children aged < 5 years, 14 d after treatment (Médecins sans Frontières-Holland [MSF-H] unpublished data). In 1998, a small study in Renk, northern Upper Nile, reported 33% treatment failures after chloroquine (NMA/UNICEF, 1999). In North Sudan, resistance to chloroquine developed faster. Recent studies report levels of around 50% in Khartoum, Gedaref, Port Sudan and Kassala (NMA/UNICEF, 1999) and Medani (Nour, 1999).

Although sulfadoxine–pyrimethamine (SP) resistance is not as high as that of chloroquine, it is rapidly increasing in East Africa where SP has replaced chloroquine as a first-line drug, i.e. in Tanzania (Rønn *et al.*, 1996) and Kenya (Nzila *et al.*, 2000a). It was not known whether resistance to SP has emerged in South Sudan. In Khartoum, surveys reported a level of SP failure of 3–11% in 1997–98 (NMA/UNICEF, 1999), whereas in Medani, 100% sensitivity was found (Nour, 1999).

Pyrimethamine and sulfadoxine are antifolate drugs that target *dhfr* and *dhps* genes, respectively. Genetic analyses have demonstrated that pyrimethamine and sulfadoxine resistance are associated with point mutations in *dhfr* and *dhps* genes (Sibley *et al.*, 2001). Resistance to SP develops with single and double *dhfr* mutations but only leads to treatment failures when parasites carry triple point mutations of *dhfr* (at codons 108, 51 and 59). The clinical resistance level increases when point mutations of *dhps* are also present (at codon 437 and/or 540, see Mberu *et al.*, 2000; Nzila *et al.*, 2000a; Mutabingwa *et al.*, 2001; Omar *et al.*, 2001; Sibley *et al.*, 2001; Kublin *et al.*, 2002).

Amodiaquine is a 4-aminoquinoline similar to chloroquine, which may remain effective where chloroquine resistance is substantial. In the past it was withdrawn from use because of rare, but severe, toxic effects when used as prophylaxis. However, a systematic review of trials revealed that amodiaquine is safe and reliable when given as a 3-d treatment (Olliaro *et al.*, 1996). It is now used in several African countries which are confronted with chloroquine resistance. In Kenya, amodiaquine was more effective than chloroquine:

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studies compiled from 1984 to 1993 revealed 86% treatment success for amodiaquine compared to 45% for chloroquine (Olliaro *et al.*, 1996), though a higher failure rate was reported more recently: 25% in Kibwezi, Kenya (Gorissen *et al.*, 2000). In Uganda, 7% resistance was found (Staedke *et al.*, 2001).

Since 1988, MSF-H has been involved in health activities in Upper Nile, southern Sudan, and is currently changing its approach from vertical towards more horizontal programmes with TB and visceral leishmaniasis treatment integrated into basic health centres. Since malaria is a major cause of illness among patients visiting the health centres, it was decided to assess the efficacy of treatment and improve the diagnosis of malaria. The current protocol to treat *Plasmodium falciparum* malaria in the Sudan is: first-line drug, chloroquine; second-line, SP; and third-line, quinine or mefloquine (NMA, 1998). Based on experience in other areas, one would expect resistance to both the first- and second-line drugs to be selected rapidly. To support better management of malaria in South Sudan, we assessed the efficacy of chloroquine, SP and amodiaquine in this area and evaluated the prevalence of mutant alleles of *dhfr* and *dhps* in patient isolates.

Patients and Methods

Study area

The study was performed from June to December 2001, in Lankien, eastern Upper Nile (Fig. 1). Lankien was considered stable enough to bring in a permanent team and equipment. MSF-H has run a visceral leishmaniasis clinic there since 1997 and Christian Mission Aid (CMA) is in charge of a primary health care centre (PHCC) in the same village. The only other health centres in the area are at 3 d and 5 d walking distance, in Waat and Malakal. Lankien has a population of 9–10 000 people, divided over 4 village tracts, totalling 614 'luaks' or households, each comprising about 15 family members (Bean, 2001). The population size of the area around Lankien is roughly estimated at 50 000–80 000. Alternating sand and black cotton soil, with acacia trees and scrubs, cover the area. In the wet season vast areas can be flooded, whereas in the dry season hardly any surface water remains. The rainy season is April to November.

Malaria transmission in Upper Nile occurs during 6 months of the wet season, May to November. Malaria is the most common disease, based on clinical diagnosis, among outpatients visiting the health centres in South Sudan (WHO/CDS/RBM, 2000). The PHCC

in Lankien, run by CMA, reported 44% of 19 500 patients as 'suspected malaria' cases from September 2000 to April 2001 (CMA, unpublished data). Data on mortality from malaria are not available. It is reported for South Sudan that 90% of malaria cases are *P. falciparum*, and 8% *P. vivax* (WHO, 2000). The vector species present are *Anopheles gambiae*, *An. arabiensis* and *An. funestus*.

Patients

All fever cases in the PHCC were brought to MSF-H for preparation of a blood slide and diagnosis of malaria. Patients selected for the study fitted the following criteria: *P. falciparum*-positive patients, aged ≥ 6 months, with fever (axillary temperature ≥ 37.5 °C) and with > 2000 asexual malaria parasites per μL . Patients with a high density of parasites ($> 100\,000$ parasites/ μL) were checked with extra care for danger signs, and included in the study when the patient appeared to be at no additional risk of developing severe malaria. Excluded were cases of mixed infections (*P. falciparum* and *P. malariae*/*P. ovale*/*P. vivax*), children aged < 6 months, pregnant women, patients with signs of severe malaria or with another febrile disease requiring treatment, patients who had taken antimalarials in the preceding 48 h, patients with a history of side effects to chloroquine or sulfa-drugs, or those living too far from the clinic to return for follow-up and not able to stay in the village. Patients or their parent/carers were given standard information and gave verbal informed consent. Patients were stratified per age group (< 5 years, 5–15 years and ≥ 15 years) and randomly divided in to 3 intervention groups: (i) 3-d treatment with chloroquine, 25 mg/kg (range 25 to ≤ 30 mg/kg), 10 mg/kg on day 0 and day 1 and 5 mg/kg on day 2; (ii) 1-d treatment with SP, 25 mg/kg sulfadoxine (25 to < 42 mg/kg) and 1.25 mg/kg pyrimethamine (1.25 to < 2.1 mg/kg) on day 0; and (iii) 3-d amodiaquine, 25 mg/kg (25 to ≤ 30 mg/kg), 10 mg/kg on day 0 and day 1 and 5 mg/kg on day 2. Observed treatment was given and if vomiting occurred within 30 min the full dose was repeated, half the dose was repeated if vomiting occurred after 30 min but within 1 h after treatment. Patients were asked to return on days 3, 7 and 14 or any day when they had fever or other signs of disease. The study was approved by an ethical committee of MSF-H, Amsterdam. The local authorities and their counterparts agreed with the study and helped to notify the population.

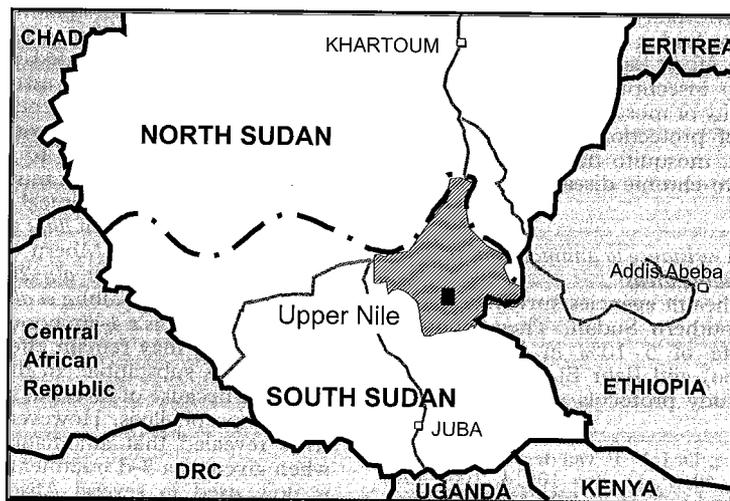


Fig. 1. Map of Sudan, showing Upper Nile with Lankien (black rectangle).

Laboratory methods

Thick and thin blood films were collected at each patient's visit. Slides were Giemsa-stained, at least 100 fields of the thick film were screened and results confirmed in the thin film. *Plasmodium falciparum*-positive slides were examined for parasite density, assuming a white blood cell count of 8000/ μL (WHO, 1991). External cross-checks (100 slides, African Medical and Research Foundation, Nairobi) revealed a sensitivity of 100% and a specificity of 95%. Internal cross-checks by senior laboratory technicians of the output of the trainee microscopists (292 slides) gave an overall error percentage of 3.4%. All errors were corrected before data processing. The level of haemoglobin (Hb) in the blood was measured on day 0 and day 14, using a BMS Hb meter.

Blood spots on filter-paper ($\pm 50 \mu\text{L}$) were collected, dried and kept at ambient temperature until analysed. Parasite genomic material was prepared using methanol fixation described by Nzila *et al.* (2000a, 2000b). Point mutations in codons 108, 51, 59 and 164 of *dhfr* and those in codons 436, 437, 540, 581 and 613 of *dhps* were analysed by polymerase chain reaction (PCR) amplification and restriction enzyme digestion (Nzila *et al.*, 2000a, 2000b).

Assessment of resistance

The main outcome variables were the proportions of treatment failures at day 3 and at day 14 for each of the 3 treatment groups. Following WHO (1996) and EANMAT (1999) guidelines treatment failures were classified in to 2 categories. Early treatment failure was reported when (i) the patient had parasites in the blood and showed signs of severe malaria on day 3 or earlier; (ii) the parasite density on day 2 was higher than on day 0; (iii) the parasite density on day 3 was $> 25\%$ of that on day 0; or (iv) the patient had parasites and fever (axillary temperature $\geq 37.5^\circ\text{C}$) on day 3. Late treatment failure was recorded when the patient showed parasitaemia and fever at any day after day 3. Patients with treatment failures after chloroquine or amodiaquine treatment were given SP. In case of SP failure or when complications arose, patients were treated as severe malaria (quinine injection), and admitted. Clinical success was recorded when no failure occurred during the patient's follow-up period. Patients with parasitaemia but no fever on day 14 were regarded as parasitological, but not clinical, failures and retreated as above.

Statistical analysis

The sample size was calculated on the basis of expected levels of efficacy of chloroquine at 75%, and SP and amodiaquine at 90%. To detect a difference of 15% or more, with 80% power at a 5% significance level, 97 patients per treatment group were needed (Campbell & Machin, 1999). Hence a total of 350 patients was needed to complete the study, taking into account a drop-out rate of 20%. Data were recorded on patient-clinic-forms and laboratory-forms, and were analysed with Excel (Microsoft Corp., Seattle, WA, USA) SPSS (SPSS Inc., Chicago IL, USA) and Epi Info (CDC, Atlanta, GA, USA). Parasite densities were normalized by logarithmic transformation. Proportions were compared using χ^2 tests with Yates's correction, or Fisher's exact test. Analysis of variance (ANOVA) was used for normally distributed data and paired sample *t* tests to compare Hb values of day 0 with day 14. Two-tailed *P* values < 0.05 were considered significant.

Results

During the period June to December 2001, blood slides of 2163 fever patients were checked, of which 579 (27%) had malaria parasites. *Plasmodium falciparum* infections accounted for 575 cases, with only 2 *P. malariae* and 2 *P. ovale* infections being found. Malaria was more prevalent among children than adults: positivity rates both in children aged < 5 years (33% of 924 patients) and 5–14 years old (32% of 633) were higher than in adults (10% of 606). Parasite densities were also significantly higher in the youngest age group (geometric mean [GM] = 10 913 parasites/ μL , $P = 0.002$) and those aged 5–14 years (GM = 8736/ μL , $P = 0.025$) than in the adults (GM = 4452/ μL). Malaria was the most common disease among fever patients (27%), followed by respiratory tract infections (17%) and visceral leishmaniasis (15%).

An increase in the number of patients and malaria cases was seen mid-rainy season (August–September) and again about 1 month after the last rains (December, Fig. 2). The number of severe malaria cases was only 2–4 per month. Three children died of malaria (pneumonia co-infection).

Study patient enrolment

Of the 579 patients with malaria parasites, 342 were eligible and chose to participate in the study (Table 1). Children aged < 5 years dominated the study population as they represented the larger proportion of fever

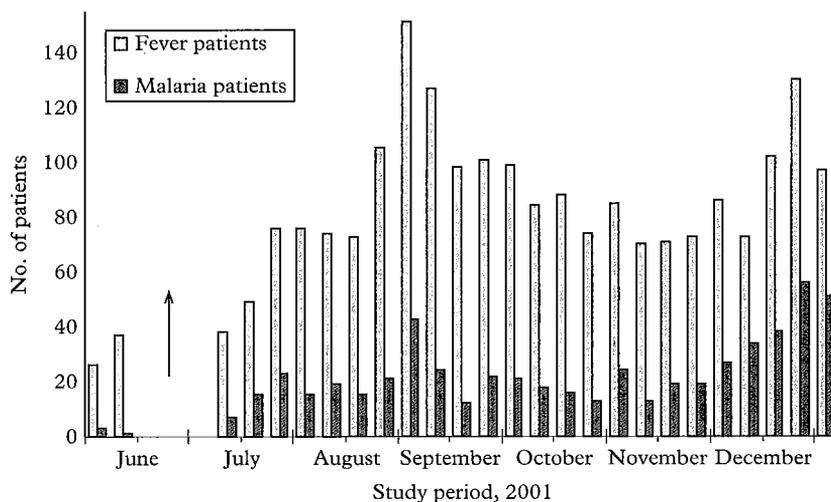


Fig. 2. Number of fever patients (axillary temperature $\geq 37.5^\circ\text{C}$) and slide-positive malaria patients seen per week at the MSF-Holland health centre, Lankien, South Sudan. Arrow indicates team evacuation due to insecurity.

Table 1. Baseline characteristics of study patients attending the MSF-Holland health centre, Lankien, South Sudan, June-December 2001

	Chloroquine (n = 121)	Sulfadoxine-pyrimethamine (n = 108)	Amodiaquine (n = 113)	Total (n = 342)
No. of females	66 (54.5%)	55 (50.9%)	63 (55.8%)	184 (53.8%)
Age (years) ^a	6.8 (0.5-50)	7.0 (0.5-39)	6.2 (0.5-37)	6.7 (0.5-50)
< 5	57 (47.1%)	57 (52.8%)	63 (55.8%)	177 (51.8%)
5-14	50 (41.3%)	39 (36.1%)	40 (35.4%)	129 (37.7%)
≥ 15	14 (11.6%)	12 (11.1%)	10 (8.8%)	36 (10.5%)
Weight (kg) ^a	19.0 (5.9-60)	19.2 (6.9-73)	17.8 (5.7-65)	18.7 (5.7-73)
Haemoglobin (g/dL) ^a	10.6 (6.5-14.8)	10.9 (5.6-14.4)	10.7 (6.0-17.0)	10.7 (5.6-17.0)
Temperature (°C) ^a	38.7 (37.5-40.6)	38.7 (35.9-40.7)	38.9 (37.5-40.5)	38.8 (35.9-40.7)
Parasite density (/ μ L) ^b	16 815 (2000-144 727)	18 034 (2039-284 480)	21 590 (2000-145 411)	18 582 (2000-284 480)

^aMean (range).^bGeometric mean (range).

patients and showed a higher positivity rate. The 3 treatment groups were equal for gender ratio, age distribution, age, weight, Hb level, temperature and parasite density. Three patients in the SP group had malaria without fever, but we decided nevertheless to keep them in the analysis. The mean Hb value was 10.7 g/dL. Twenty-seven patients (7.9%) were anaemic (Hb \leq 8.0 g/dL), of which 23 were aged < 5 years and 4 aged 5-14 years. The GM parasite density was 18 582 parasites/ μ L. Nineteen study patients had a density > 100 000 parasites/ μ L, of which 10 were aged < 5 years and 9 were aged 5-14 years. Only one of these had a density > 200 000/ μ L.

Follow-up of patients

Patients who did not return for follow-up were traced as far as possible. Thirty-five (10.2%) were lost to follow-up. The majority of patients returned on the requested day of follow-up (80%), although a few came earlier (3.7%) or later (16.3%). Patients that came early were requested to return if feeling ill and late comers were questioned about their health in the previous days. Patients who missed day 3 or day 7 follow-up were still included (12 and 9, respectively), but absentees on day 14 were considered as lost. In general, reasons for coming late or being absent were: (i) unable to leave children at home alone; (ii) illness of parent/guardian; (iii) harvesting; (iv) insecurity; and (v) visiting other areas. The latter 2 reasons were more common at the end of study, the start of the dry season, when fighting in the area resumed and the population started moving to cattle camps (permanent green areas where cattle are herded in the dry season).

Efficacy of chloroquine, SP and amodiaquine

The rate of clinical success of chloroquine treatment was 88.5%, of SP was 100% and of amodiaquine was 94.1% (Table 2). Those aged < 5 years showed the highest level of treatment failure: 16.7% in the chloroquine group and 7.0% in the amodiaquine group. Those aged 5-14 years showed intermediate levels of treatment failures to these 2 drugs, whereas in adults no treatment failures were found to any of the drugs. Of the 12 failures to chloroquine and 6 to amodiaquine, 4 and one were early treatment failures, respectively; and 4 of these were in children aged < 5 years. On day 14, 3 parasitological failures were found: 2 for chloroquine and one for amodiaquine.

Sulfadoxine-pyrimethamine was significantly more effective than both chloroquine ($P = 0.0012$) and amodiaquine ($P = 0.014$) in the overall study population. In those aged < 5 years, SP was again significantly more effective than chloroquine ($P = 0.019$). There was no significant difference between the efficacy of chloroquine and amodiaquine.

The parasitological response to treatment is shown in Fig. 3A. On day 3, apart from the early treatment failures, an additional 12 patients still had parasites in their blood but had no fever (6 for chloroquine, 3 for SP and 3 for amodiaquine). Two of these developed into a treatment failure. Similarly, of 5 patients who were parasitaemic but afebrile on day 7, 4 returned with fever at a later date. The graph in Fig. 3A extends to day 17, as 2 patients with parasites and fever were late for day 14, i.e. one for amodiaquine and one for chloroquine, but were still counted as late treatment failures, assuming they would have tested positive on day 14. In Fig. 3B the percentage of patients with gametocytes in their blood is shown. Maximum numbers were seen at day 9 (patients returning on day 7 plus those 1, 2 or 3 d late): 58% of patients treated with SP, 30% with chloroquine and 22% with amodiaquine. None of the 3 treatments effectively killed all gametocytes, but chloroquine and amodiaquine had a higher gametocidal effect than SP ($P < 0.001$, for day 9 data).

We genotyped the *dhfr* and *dhps* of 44 isolates col-

Table 2. Clinical assessment of treatment with chloroquine, sulfadoxine-pyrimethamine and amodiaquine in study patients attending the MSF-Holland health centre Lankien, South Sudan, June-December 2001

	Chloroquine (<i>n</i> = 121)		Sulfadoxine- pyrimethamine (<i>n</i> = 108)		Amodiaquine (<i>n</i> = 113)		Total (<i>n</i> = 342)
	<i>n</i> (%)	95% CI	<i>n</i> (%)	95% CI	<i>n</i> (%)	95% CI ^a	<i>n</i> (%)
Drop-out	17 (14.0)		6 (5.6)		12 (10.6)		35 (10.2)
Adequate clinical response	92 (88.5)	80.7-93.9	102 (100)	96.4-100	95 (94.1)	87.5-97.8	
Age (years)							
<5	40 (83.3)	69.8-92.5	53 (100)	93.3-100	53 (93.0)	83.0-98.1	
5-14	41 (91.1)	78.8-97.5	37 (100)	90.5-100	34 (94.4)	81.3-99.3	
≥15	11 (100)	71.5-100	12 (100)	73.5-100	8 (100)	63.1-100	
Treatment failure	12 (11.5)		0		6 (5.9)		
Early treatment failure	4 (3.8)		0		1 (1.0)		
Late treatment failure	8 (7.7)		0		5 (4.9)		
Parasitological failure	2 (1.9)		0		1 (1.0)		

^a95% CI values are exact, based on the binomial distribution.

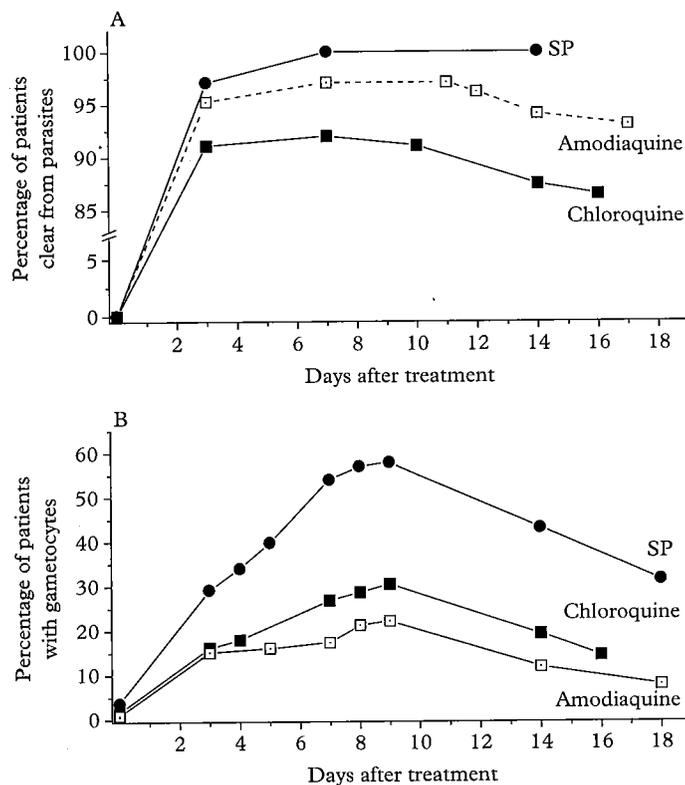


Fig. 3. (A) Parasitological response and (B) gametocidal response to three treatment regimens in study patients attending the MSF-Holland health centre, Lankien, South Sudan, June-December 2001.

lected before SP treatment. As mentioned previously, all patients were aparasitaemic up to day 14 after SP use. For *dhfr*, 36/44 (82%) were wild type, 1/44 (2%) was a single mutant (at codon 108) and 7/44 (16%) were double mutants (at codons 108 and 51 or 108 and 59). The *dhps* genotyping showed that all 44 isolates were wild-type. Parasites with such genotypes have been reported previously as clinically sensitive to SP treatment, and this was confirmed in our study.

The Hb level showed a small but significant increase after treatment with chloroquine, from 10.7 g/dL before to 11.1 g/dL after treatment ($P = 0.028$) and SP (10.9 to 11.3 g/dL, $P = 0.013$), but not with amodiaquine (10.9 to 11.1 g/dL, $P = 0.15$). No severe side effects were reported for any of the 3 treatments.

Symptoms such as headache, dizziness, vomiting and anorexia were reported after treatment in all 3 groups, but these most probably related to the malaria infection itself. Skin rash and itching was reported after chloroquine and amodiaquine treatment. Thirty-five patients received additional treatment during their follow-up visits (20 for respiratory tract infections, 8 for worms, 3 for ear infections, 2 for eye infections and 2 for diarrhoea).

Discussion

The incidence of malaria among fever patients in Lankien, Upper Nile, South Sudan, was lower than predicted from data from health agencies working in the area. On average, about one-quarter of fever

patients had malaria parasites in the blood. In Lankien, *P. falciparum* was responsible for 99.3% of infections. The main malaria peak occurred about 1 month after the rainy season, in December.

Lankien may be a locality of lower malaria endemicity within the Upper Nile region which has a presumed malaria intensity of stable, hyperendemic nature (El Gadal, 1991; MARA/AMRA 1998). We found that at the end of the dry season malaria prevalence was low (10% of fever patients), rose gradually during the wet season and peaked after the rains (up to 50% of fever patients). Children aged < 5 years are generally considered the most vulnerable for malaria. According to our findings in Lankien, children aged 5–14 years still appear to be at high risk for malaria, as they showed a positivity rate similar to that of those aged < 5 years. Parasite densities in both these age groups were higher than in adults. Most of the patients with anaemia were aged < 5 years (23/27), although this may be related to other factors apart from malaria, such as malnutrition.

This study shows that *P. falciparum* strains in eastern Upper Nile, South Sudan, are still more sensitive to antimalarials than in surrounding countries and other regions in Sudan. We found 12% treatment failure after chloroquine and 6% failure after amodiaquine treatment in patients of all ages, during the 14-d follow-up, whereas SP was completely effective. The chloroquine failure rate was higher in children aged < 5 years, who are generally considered to be the most important group in terms of assessment of efficacy of antimalarial treatment. In 17% of these chloroquine failed to cure the infection or malaria parasites and symptoms returned within the 14-d study period. Levels of treatment failure in those aged 5–14 years were intermediate, whereas no recrudescence infections were found in our small group of 36 adults.

Chloroquine resistance is still below the level of 25%, the level at which the WHO advises changes to the treatment protocol (Bloland *et al.*, 1998). Recently, WHO stated that a level of 6–15% treatment failures should alert decision makers, and at a level of 16–24% failures the process of changing protocols should be initiated (WHO, 2002). Hence the figure of 17% resistance in children aged < 5 years in Lankien is important. This study only gives information about the eastern Upper Nile area. In other areas of South Sudan resistance may have developed at a faster rate. Chloroquine resistance generally invades an area by the spread of resistant strains from other areas, rather than arising *de novo* (Hastings, 2001). Lankien is about 120 km from the Ethiopian border and some travelling occurs between the 2 countries, but the prevalence of chloroquine resistance in Ethiopia appears to be low and confined to the central part of the country (Alene & Bennet, 1996). In areas which are nearer to the Kenyan/Ugandan borders, a higher level of resistance to chloroquine can be expected.

Amodiaquine did not perform significantly better than chloroquine in this study. This drug has not been used in Upper Nile before; therefore the apparent treatment failures probably arise from (partial) cross-resistance between amodiaquine and chloroquine (Olliaro *et al.*, 1996). Amodiaquine at a total dosage of 30 or 35 mg/kg might have given better results (P. Ringwald, personal communication). It seems unnecessary to introduce amodiaquine as a 'new' drug in South Sudan. It can be confused with chloroquine among health workers because although dosages of amodiaquine are similar to chloroquine, the tablets contain different amounts of active compound.

Sulfadoxine-pyrimethamine is still completely clinically effective. This is in agreement with the results of genotyping the 2 genes that are associated with SP resistance: more than 80% and 100% of parasites were wild type *dhfr* and *dhps*, respectively. Not a single isolate carried alleles with the 3 point mutations at 108,

51 and 59 (triple mutant), an early marker of SP resistance. This supports the high efficacy of SP described in this report. However, SP has an extremely long half-life within the patient (Watkins & Mosobo, 1993; Nzila *et al.*, 2000b) and experience from other malaria-endemic areas has shown that widespread use of SP as monotherapy is likely to lead to rapid selection of triple mutant *dhfr* alleles and double mutants in *dhps* (Sibley *et al.*, 2001).

Based on our findings presented here, we recommend the timely introduction of combination treatment in this area. This should delay the emergence of highly resistant parasites and prolong the therapeutic life of the drugs available (White *et al.*, 1999; Hastings, 2001). In addition to this, the vulnerability of this population living in a chronic emergency situation with poor access to health care, necessitates a completely effective first-line treatment.

The best option is a combination treatment of SP and artesunate. Artesunate is a fast-acting antimalarial, which causes a rapid clearance of parasites from the blood, has a gametocidal action and thus can lower the rate of transmission (Price *et al.*, 1996). Nowadays, WHO and MSF advocate artesunate-based combination therapy (ACT) in endemic countries where resistance to currently used drugs has reached the threshold of 15%. Since the price of artesunate is decreasing rapidly, the financial obstacle to its introduction is reduced. This study in eastern Upper Nile indicates that SP is a suitable companion drug. Artesunate-SP combination treatment has proven to be very beneficial in the treatment of uncomplicated malaria (Von Seidlein *et al.*, 2000). A change in protocols towards ACT in South Sudan should be discussed and coordinated among NGOs, local authorities and any others interested. More studies in other regions in South Sudan are required. The introduction of ACT should go hand in hand with laboratory-confirmed diagnosis (microscopy or rapid diagnostic tests) to prevent unnecessary use of valuable drugs.

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