Tropical Medicine and International Health

VOLUME 10 NO 2 PP 139–145 FEBRUARY 2005

Efficacy and effectiveness of the combination of sulfadoxine/pyrimethamine and a 3-day course of artesunate for the treatment of uncomplicated falciparum malaria in a refugee settlement in Zambia

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Summary In the Maheba Refugee Settlement, in the clinics supported by Médecins Sans Frontières, all children aged up to 5 years with a confirmed diagnosis of uncomplicated falciparum malaria are treated with the combination of sulfadoxine/pyrimethamine (SP) and artesunate (AS). We compared the treatment's efficacy and effectiveness. Patients were randomized in order to receive the treatment supervised (efficacy) or unsupervised (effectiveness). Therapeutic response was determined after 28 days of follow up. The difference between recrudescence and re-infection was ascertained by polymerase chain reaction (PCR). We also assessed genetic markers associated to SP resistance (*dhfr* and *dhps*). Eighty-five patients received treatment under supervision and 84 received it unsupervised. On day 28, and after PCR adjustment, efficacy was found to be 83.5% (95% CI: 74.1–90.5), and effectiveness 63.4% (95% CI: 52.6–73.3) ($P < 0.01$). Point mutations on *dhfr* (108) and *dhps* (437) were found for 92.0% and 44.2% respectively of the PCR samples analysed. The significant difference in therapeutic response after supervised and unsupervised treatment intake can only be explained by insufficient patient adherence. When implementing new malaria treatment policies, serious investment in ensuring patient adherence is essential to ascertain the effectiveness of the new treatment schedules.

keywords malaria, *Plasmodium falciparum*, efficacy, effectiveness, artemisinin-based combination therapy, refugee settlement, Zambia

Introduction

The high burden of malaria on the African continent, aggravated by the increasing spread of parasites resistant to the currently used antimalarial drugs, is well-documented (White *et al.* 1999; Bloland 2001; Trape 2001). One strategy to improve cure rates and limit the development of drug resistance is the use of artemisinin-based combination therapy (ACT) (White & Olliaro 1996; White 1999; Guerin *et al.* 2002). A meta-analysis including almost 6000 patients showed higher cure rates, faster parasite clearance and a decreased gametocyte carriage with ACT than monotherapy (International Artemisinin Study Group 2004). The introduction of ACT implies that the long treatment course of chloroquine or the convenient single dose of sulfadoxine-pyrimethamine (SP) are replaced by new 3-day course therapies. However, poor patient adherence to such novel protocols would impair treatment effectiveness, and favour the development of resistance (Nosten & Brasseur 2002). In Zambia, resistance has been measured at 54% for chloroquine, and between 16 and 26% for SP (Bijl *et al.* 2000; National Malaria Control Centre 2000). In November 2001, in the Maheba Refugee Settlement (North-Western Province), Médecins Sans Frontières (MSF) introduced the combination of SP single dose and 3 days artesunate (AS), for the treatment of uncomplicated falciparum malaria, confirmed by a rapid diagnostic test (Paracheck Pf®, Orchid Biomedical System, Goa, India), in children of 5 years or younger. After 3 months of using this new protocol, assessment of patient adherence revealed that 21% of the patients had leftover tablets on the day after the last treatment dose, and that

another 39% had completed the treatment but with incorrect timing and/or dosage (Depoortere *et al.* 2004). In order to assess the impact of this limited adherence, we compared the treatment efficacy and effectiveness, i.e. the therapeutic response after supervised and unsupervised drug intake, respectively.

Materials and methods

Study area and population

Patients were recruited in clinic H of the Maheba Refugee Settlement, the characteristics of which are described elsewhere (Depoortere *et al.* 2004). Eligible for study participation were children between 6 and 59 months of age, weighing at least 5 kg, with fever (axillary temperature ≥37.5 °C) or history of fever in the past 24 h and with a microscopically confirmed monoinfection of *Plasmodium falciparum* (parasitaemia ≥2000/μl and <100 000/μl). Children with danger signs or signs of severe malaria according to the World Health Organisation (WHO) criteria, with a history of allergy to SP or living at more than 1.5 h walking distance from the health clinic were excluded from study participation (WHO 1996, 2002).

Enrolment procedure

Patients suspected with malaria during the outpatient consultation were sent to the malaria study clinic. After clinical examination, a thick and thin smear was taken and haemoglobin measured. For patients fulfilling all inclusion/exclusion criteria, written informed consent was obtained. Randomized in blocks of 10, children were assigned to be given treatment either in a supervised or unsupervised way. Before receiving any treatment, a blood sample was taken on filter paper for nested polymerase chain reaction (PCR) analysis.

Treatment

The treatment schedule to be evaluated consisted of a single dose SP (sulfadoxine 500 mg and pyrimethamine 25 mg, International Dispensary Association, the Netherlands; based on 25 mg sulfadoxine/kg) and 3 days AS (artesunate 50 mg, Dafra Pharma nv, Belgium; 1 dose/day, 4 mg/kg/day). Children in the supervised arm were given the first dose at the health clinic, and were asked to return on the following 2 days for observed intake of the whole treatment. The supervised doses were given with great care, ensuring that no medication was spilled, spit or vomited; for the smallest children, tablets were crushed and given by means of a spoon or a syringe. In case vomiting occurred, a second attempt to give the treatment was made after 30–60 min. When the child vomited any of the supervised doses more than once, intravenous rescue treatment was given [artemether (3.2 mg/kg/day the first day, and 1.6 mg/kg/day the second and the third days), or quinine hydrochloride (10 mg/kg/8 h for 7 days)]. Children in the unsupervised arm were prescribed the SP/AS combination on their health cards, to receive the complete treatment at the pharmacy, with explanation for home administration, as was done under usual circumstances. No further measures were undertaken for the treatment of these patients.

Patient follow up

Follow up was done according to WHO guidelines (WHO 1996, 2002). Visits for both groups were scheduled on day 3, 7, 14, 21 and 28, during which a clinical and parasitological examination was conducted. In case of parasitaemia after day 9, a second PCR sample was taken. The presence of gametocytes was assessed on day 14 and 28, and haemoglobin levels were measured on day 28. If perceived necessary, patients were free to attend the malaria study clinic at any time during follow up. Children were withdrawn from the study in case of failure to take any of the supervised doses, vomiting any of the supervised dose more than once, danger signs or severe disease, self-medication with any antimalarial, decision of the parent or guardian, or mistaken inclusion. Patients for whom the outcome could not be determined were considered lost to follow up.

Classification of therapeutic outcomes

Following WHO criteria for intense transmission areas, early treatment failure (ETF) was defined as (i) signs of severe malaria with parasitaemia on day 1, 2 or 3; or (ii) parasitaemia on day 2 exceeding parasitaemia of day 0 combined with fever; or (iii) parasitaemia on day 3 exceeding 25% of parasitaemia of day 0; or (iv) fever and parasitaemia on day 3 (WHO 2002). Late clinical failure (LCF) was defined as any parasitaemia between day 4 and day 28 in the presence of fever. Patients were classified as late parasitological failure (LPF) when they presented positive parasitaemia on day 28, but without any clinical signs. Finally, patients showing negative parasitaemia on day 28 were classified as adequate clinical and parasitological response (ACPR). Children classified as ETF or LCF during the follow-up period, as well as children classified as LPF on day 28, received oral quinine hydrochloride as rescue treatment (10 mg/kg/8 h for 7 days).

Laboratory procedures

A thin and thick smear were prepared on the same slide and stained using 10% Giemsa solution. Each blood slide was read blindly by two technicians. In case of disagreement, the reading of a third technician would decide on the final result. We also had external quality control. Parasitaemia was quantified by counting asexual parasites against 200 white blood cells (WBCs) and assuming a normal WBC level of 8000/μl. Presence of gametocytes was ascertained counting 200 WBCs, or 500 WBCs in case no trophozoites were seen. Haemoglobin levels were measured by means of a Lovibond®. Samples for PCR genotyping were collected on Isocode^{TM} Stix (Schleicher & Schuell, Dassel, Germany), on day 0 for every patient included, and a second for every child presenting with positive parasitaemia after day 9. *In vitro* resistance to SP was assessed through the prevalence on day 0 of point mutations on codon 108 on the *dhfr* gene, and on codon 437 on the *dhps* gene (Kublin *et al.* 2002). Recrudescence was distinguished from re-infection by comparison of *msa-2* gene polymorphism (Contamin *et al.* 1995). DNA extraction, seminested PCR followed by restriction fragment length polymorphism (RFLP) methods using specific enzymes were performed at the Laboratoire de Parasitologie Médicale, Faculté de Médecine, in Rouen (France) according to published protocols (Vasconcelos *et al.* 2000).

Sample size

Considering the data on SP resistance in Zambia on one hand, and comparisons of AS efficacy in monotherapy *vs.* the combination of AS/SP on the other, efficacy was estimated at 90% (Von Seidlein *et al.* 2000). Taking into account the results of the adherence study on the same site a little earlier, effectiveness was estimated at 70% (Depoortere *et al.* 2004). To detect this 20% difference, with a type 1 error of 0.05 and a power of 80%, 70 children were required in each treatment arm. Planning for a 20% loss to follow up, the total sample size was estimated at 168 children.

Statistical analysis

Data were daily entered using Microsoft Excel® (Office 2000), and analysed using EPI-INFO 6.04 software (CDC, Atlanta, USA) and SPSS® version 10.0.5 (SPSS Inc., Chicago, IL, USA). All data were individually checked for incoherencies. Treatment outcomes are expressed as proportions of the total analysable results. Mean values for temperature and haemoglobin concentration were compared using *t*-distribution and ANOVA test statistic. Comparisons between proportions were done using the chi-square test and presented with a 95% confidence interval and *P*-value.

Ethics

The study protocol was approved by the Zambian Research Ethics Committee.

Results

Study profile and baseline characteristics

The study was conducted between March and June 2002. Of 333 patients screened, 169 were included: 85 (50.3%) were randomized in the supervised arm, and 84 (49.7%) in the unsupervised arm (Figure 1). None of the patients reported intake of antimalarial treatment before the consultation. There was no difference in baseline characteristics between the two treatment arms (Table 1). Eight patients (4.7%) were withdrawn from the study, six (7.0%) in the supervised group (three for vomiting and three for self-medication) and two (2.4%) in the unsupervised group (one for vomiting and one for self-medication) ($P = 0.28$). None of the patients was lost to follow up.

Therapeutic response

On day 14, treatment efficacy was estimated at 86.6% (95% CI: 77.9–92.7) and treatment effectiveness at 71.9% (95% CI: 61.5–80.9) ($P = 0.02$). On day 28, and after PCR adjustment, treatment efficacy was 83.5% (95% CI: 74.1–90.5) and its effectiveness 63.4% (95% CI: 52.6–73.3) ($P = 0.004$) (Table 2). There was no difference between the supervised and unsupervised group in the proportion of patients with fever on day 0 (50.6% *vs.* 57.8% respectively; $P = 0.35$) or day 3 (4.8% *vs.* 2.5%; $P = 0.70$). There was no significant difference either in the proportion of patients with gametocytes on day 0 (11.8% *vs.* 19.0%; $P = 0.19$) or during follow up on day 14 (4.8% *vs.* 4.9%; $P = 0.74$) and day 28 (5.9% *vs.* 8.3%; $P = 0.84$). Lastly, for the 62 children in the supervised group anaemic on day 0 (defined as haemoglobin <11 g/dl), the anaemia had disappeared on day 28 for 30 of them (48.4%). In the unsupervised group, anaemia disappeared for 25 of 56 children (44.6%) ($P = 0.68$).

Genomic analysis

All 113 PCR samples from day 0 were analysed for mutations related to SP resistance. Point mutations on

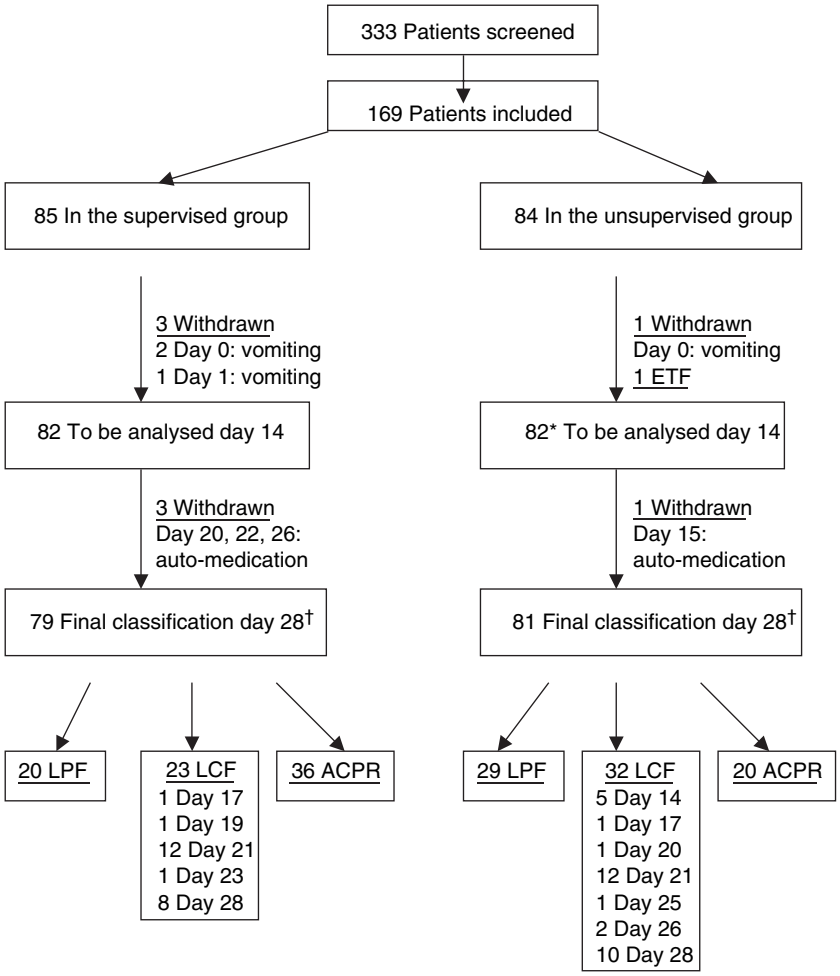

Figure 1 Study profile. Clinic H, Maheba, Zambia (March–June 2002).
* One patient did not show up on day 14 and could therefore not be included in the day 14 analysis. † Results before polymerase chain reaction (PCR) adjustment.

Table 1 Socio-demographic and clinical characteristics of patients on inclusion (clinic H, Maheba, Zambia, March–June 2002)

	Supervised ($n = 85$)	Unsupervised ($n = 84$)	*P*-value
Mean age (months) (SD)	23.6 (12.5)	21.4 (13.4)	0.27
Mean weight (kg) (SD)	9.5 (2.0)	9.5 (2.4)	0.93
Gender ratio (M/F)	0.81 (38/47)	0.91 (40/44)	0.76
Mean temperature (°C) (SD)	37.6 (1.1)	37.8 (1.2)	0.26
Median haemoglobin (g/dl) (range)	9.2 (5.3–12.2)	9.4 (5.3–12.7)	0.46
Geometric mean parasitaemia (range)	16 022 (2224–90 435)	19 185 (2185–97 953)	0.41
Gametocytes	10 (11.8%)	16 (19.0%)	0.19

codon *dhfr* (108) were seen in 92.0% (95% CI: 85.4–96.3) of the samples on inclusion (90.4% in the supervised group, 93.4% in the unsupervised group, $P = 0.80$). On codon *dhps* (437), point mutations were present in 44.2% (95% CI: 34.9–53.9) of the samples, 36.5% in the supervised, and 50.8% in the unsupervised group ($P = 0.12$) (Table 3). In patients that were finally classified as treatment failures, there was no statistically significant

Table 2 Therapeutic response at day 14 and day 28 of both supervised and unsupervised treatment groups, corrected by PCR (clinic H, Maheba, Zambia, March–June 2002)

	Supervised			Unsupervised		
	n	%	95% CI	*n*	%	95% CI
Outcome at day 14						
Early treatment failure	0	0.0	0.0–3.6	1	1.2	0.1–5.9
Late clinical failure	0	0.0	0.0–3.6	5	6.1	2.3–13.0
Late parasitological failure	11*	13.4	7.3–22.1	17	20.7	13.0–30.5
Adequate clinical and parasitological response	71	86.6	77.9–92.7	59	71.9	61.5–80.9
Total	82	100.0		82	100.0	
Outcome at day 28						
Early treatment failure	0	0.0	0.0–3.6	1	1.2	0.1–5.9
Late clinical failure	6	7.6	3.1–15.1	13	15.9	9.1–25.0
Late parasitological failure	7	8.9	3.9–16.7	16	19.5	12.0–29.1
Adequate clinical and parasitological response	66	83.5	74.1–90.5	52	63.4	52.6–73.3
Total	79	100.0		82	100.0	

PCR, polymerase chain reaction; CI, confidence interval.
* One patient came on day 15 for follow up, instead of on day 14.

difference between the two treatment arms in the presence of point mutations. For *dhfr* (108), in the supervised arm there were 27.7% of the patients with a point mutation and 41.8% in the unsupervised arm ($P = 0.14$). For *dhps* (437), these proportions were 10.6% and 25.4% respectively ($P = 0.05$). When considering the mixed profiles as resistant as well (mutations are selected after SP treatment), proportions became 97.3% (95% CI: 92.4–99.4) and 59.3% (95% CI: 49.6–68.4), respectively.

Discussion

We compared the efficacy and effectiveness of the combination of SP and AS in children under 5 years of age. After 14 and 28 days of follow up, we found that, in Maheba, cure rates obtained with the combination AS/SP were significantly lower when drugs were given under normal field conditions. The difference between the two groups might have been slightly overestimated: while in the efficacy group each dose intake was supervised and vomiting systematically led to exclusion, in the unsupervised group exclusion happened only if vomiting occurred during the inclusion process. However, since only three children had to be excluded in the supervised group, and since randomization did not show any difference between the two groups on inclusion, we strongly believe this had limited influence on our results. No difference between the two treatment groups was found for the clearance of fever and parasites on day 3, disappearance of gametocytes on day 14 and 28, or improvement of haemoglobin levels on day 28. We have no knowledge of previous studies that investigated the effectiveness of ACT after a 28-day follow up and with PCR adjustment, in routine conditions, to compare with our results.

Table 3 Prevalence of point mutations on the *dhfr* and *dhps* genes (day 0 samples; clinic H, Maheba, Zambia, March–June 2002)

	n	%	95% CI
dhfr (108)			
Resistant	104	92.0	85.4–96.3
Mixed	6	5.3	2.0–11.2
Sensitive	3	2.7	0.5–7.6
Total	113	100.0	
dhps (437)			
Resistant	50	44.3	34.9–53.9
Mixed	17	15.0	9.0–23.0
Sensitive	46	40.7	31.6–50.3
Total	113	100.0	

The reduced efficacy of AS/SP we found (83.5% after 28 days) is most likely due to the high resistance levels to SP. In 1998 and 1999, *in vivo* studies estimated SP resistance in two different regions of the country at 26 and 16% (Bijl *et al.* 2000; National Malaria Control Centre 2000). The high proportion of genetic mutations in Maheba, linked to pyrimethamine and sulfadoxine resistance, suggests that resistance levels might be even higher than these estimates. However, because only one point mutation was considered on both genes (possibly leading to an overestimation of resistance levels), true resistance probably lies somewhere between the *in vivo* and genetic results (Peterson *et al.* 1988; Omar *et al.* 2001; Kublin *et al.* 2002). In any case, these results suggest that the use of SP, even combined to an artemisinine derivative, is no

longer indicated in Maheba. At the time of our study, the Zambian government was considering to change national policy, which prescribed chloroquine as first-line treatment. Country policy now prescribes artemether-lumefantrine.

The observed drop in cure rates after unsupervised drug intake, from 83.5 to 63.4%, is likely to be mainly, if not completely, because of poor patient adherence. The previously completed adherence study indicates the same assumption. Our results underline the significant implications of non-adherence, particularly in case of moderate efficacy. When preparing changes in malaria treatment policy, decisions are usually based on treatment efficacy measures only. However, more attention should be paid to the testing of new treatment protocols in true field conditions. We strongly believe that ACT is the only alternative for effective treatment of *P. falciparum* malaria today. However, serious investment is needed to monitor and improve patient adherence to treatment, in order to ensure that the drugs truly *cure* the patients.

Acknowledgements

The authors kindly thank the local study team for their dedication: Gilbert Siame, Thacien Munyanshongore, Custodia Mateus, Frank Nyimbili, Winford Kayeyi, Alfonso Ndielo, Augusto Kapalo, Shadrick Ndong'u, José Antunes Ngola, Owen Murhambo and Augustino Kachambelele.

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