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Antimalarial efficacy of chloroquine, amodiaquine, sulfadoxine-pyrimethamine, and the combinations of amodiaquine + artesunate and sulfadoxine-pyrimethamine + artesunate in Huambo and Bié provinces, central Angola

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Angola

Summary We studied three antimalarial treatments in Caala and Kuito, Angola, in 2002 and 2003. We tested chloroquine (CQ), amodiaquine (AQ) and sulfadoxine-pyrimethamine (SP) in Caala, and AQ, SP and the combinations AQ + artesunate (AQ + AS) and SP + artesunate (SP + AS) in Kuito. A total of 619 children (240 in Caala, 379 in Kuito) with uncomplicated *Plasmodium falciparum* malaria were followed-up for 28 days, with PCR genotyping to distinguish recrudescence from reinfection. PCR-corrected failure proportions at day 28 were very high in the CQ group (83.5%, 95% CI 74.1—90.5), high in the SP groups (Caala: 25.3%, 95% CI 16.7—35.8; Kuito: 38.8%, 95% CI 28.4—50.0), around 20% in the AQ groups (Caala: 17.3%, 95% CI 10.0—27.2; Kuito: 21.6%, 95% CI 14.3—30.6) and very low in the artemisinin-based combination groups

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(1.2%, 95% CI 0.0—6.4 for each combination AQ + AS and SP + AS). These results show that CQ and SP are no longer efficacious in Caala and Kuito and that the moderate efficacy of AQ is likely to be compromised in the short term if used as monotherapy. We recommend the use of AQ with AS, though this combination might not have a long useful therapeutic life because of AQ resistance.

1. Introduction

In Africa, *Plasmodium falciparum* resistance to common antimalarials represents a major obstacle for malaria control. In the Republic of Angola, which reports 1.5 million clinical cases of malaria each year (MINSA, 2001), the development of resistance to first-(chloroquine, CQ) and second-line (sulfadoxine-pyrimethamine, SP) treatments represents an important problem for the national health authorities. The first cases of resistance of *P. falciparum* to CQ and SP in Angola were reported almost 20 years ago (Martinez et al., 1985; Peña et al., 1988). Additional data were collected during the 1980s and 1990s (Kyronseppa et al., 1984; Laureillard et al., 1996; Lindberg et al., 1985; Suleimanov, 1994). However, these data were difficult to interpret and compare as different study designs had been used. Although observations from local clinicians suggested that CQ and SP were losing their efficacy, no recent data on antimalarial drug efficacy were available to the Angolan Malaria Control Program for the formulation of a sound national drug policy. In 2001, in order to fill this gap and explore potential alternatives to the current policy, the Ministry of Health carried out several in vivo studies in Luanda, Cabinda, Malange, Huambo, Bié, Benguela and Huila provinces. At three sites, the studies were carried out in collaboration with Medecins sans Frontiéres (MSF). In this paper, we report the results obtained from two of these sites (Caala, Huambo province; Kuito, Bié province) where, unlike the other sites, the follow-up was extended to 28 days and artemisinin-containing treatments (ACT) were evaluated. The protocols were discussed with a Scientific Committee of the Ministry of Health (MOH) in Luanda and eventually approved by the national and provincial health authorities. Specific changes introduced to our study protocols in Caala and Kuito (duration of 28 days, use of PCR for genomic analysis, use of ACT) were also approved by the MOH at national and provincial level.

2. Materials and methods

2.1. Study site and population

The studies were conducted in the towns of Caala and Kuito, located in central Angola. Both areas have been heavily affected by the civil war that divided the country for more than two decades. This explains the deterioration of roads and buildings, the lack of basic services (water, sanitation and electricity), the limited communications with the rest of the country, and the presence of a large internally displaced population (IDP), residing in camps, whose access to health care has been largely insufficient. Medecins sans Frontiéres has been present in Kuito since 1989 and in Caala since 1994, working in IDP camps and supporting the hospitals of the towns. Huambo and Bié provinces have been classified as mesoendemic, with malaria being stable and seasonal with a peak of transmission from September to April. Annual temperatures range from 21 to 26 °C and annual rainfall between 200 and 1000 mm (MINSA, 2001). In Caala, patients were recruited from Caala Health Centre where malaria represents an important cause for attendance: between January and April 2002, 5220 cases of malaria diagnosed on clinical bases were reported, representing 24% of the total consultations. In Kuito, patients were recruited from the Out Patient Department (OPD) of Bié Provincial Hospital. Four weeks after the start of the study and because of the low recruitment, patients were also recruited from three health posts and one Maternal and Child Centre, all located within a 10 km distance of the hospital.

2.2. Inclusion criteria and procedures

The studies were based on current WHO recommendations (WHO, 2002) and conducted in accordance with the ethical principles contained in the Declaration of Helsinki. In brief, children aged 6 to 59 months, living within 1 hour's walk of the clinic,

weighing more than 5 kg and presenting with measured fever (axillary temperature $\geq$37.5 °C) (Kuito) or history of fever in the past 24 h (Caala) were screened and sent to the study clinic. In Kuito, a preliminary Paracheck-Pf® (Orchid Biomedical System, India) rapid test was also performed when peripheral facilities were included so as to minimize unnecessary travel to the study clinic in Kuito town. Children with a *P. falciparum* monoinfection and asexual parasitaemia between 2000 and 100 000/μl were eligible for the study. Exclusion criteria were (i) signs of severity or severe malaria including severe anaemia defined by haemoglobin <5 g/dl (WHO, 2000); (ii) reported intake of a full course of antimalarials in the previous 7 days; (iii) history of allergic reactions to the study drug; (iv) presence of a concomitant febrile condition with the potential to confound study outcome; and (v) severe malnutrition. Written, informed consent explained in Portuguese or the local language (Umbundo) was obtained from parents or guardians.

2.3. Treatment and follow-up

Study regimens consisted of SP 1.25 mg/kg stat (in Caala: Laboratoires Creat, France; in Kuito: Fansidar®, Roche, France); amodiaquine (AQ) 30 mg/kg base divided into three daily doses of 10 mg/kg (Camoquin®, Parke-Davis, Senegal); CQ 25 mg/kg base given at 10 mg/kg on day 0 and day 1, and 5 mg/kg on day 2 (IDA, Netherlands); and artesunate (AS) at a daily dose of 4 mg/kg on days 0, 1 and 2 (Arsumax®, Sanofi Winthrop, France). In both sites, drugs were crushed and mixed with water and sugar, given in a spoon or a syringe in smaller children. All doses were directly observed and repeated if vomiting occurred within 30 min.

In Caala, the study was conducted between April and June 2002. Patients were randomly allocated in blocks of 20, without a concealment procedure, to receive either CQ or AQ, whereas patients in the SP group were recruited after the end of allocation to the CQ and AQ groups. In Kuito, SP and AQ were evaluated sequentially between October 2002 and February 2003 (SP first, AQ immediately after). The combinations AQ + AS and SP + AS were evaluated between March and July 2003, after the end of the SP and AQ groups. Patients were randomly allocated in blocks of 20, again without a concealment procedure, to receive either AQ + AS or SP + AS, although due to technical problems the randomization started only after 30 AQ + AS patients had been included.

On day 0, blood samples were taken for haemoglobin measurement, parasitaemia including gametocytes (Kuito) and genotyping analysis to distinguish recrudescence from reinfection. After treatment (days 0, 1, and 2), children were reassessed clinically and parasitologically on days 3, 7, 14, 21 and 28. If children were parasitaemic but asymptomatic during follow-up, additional home visits were performed or patients were asked to return to the clinic every 3 days (according to WHO guidelines, treatment is withheld for such children until either appearance of symptoms or day 28). Haemoglobin was reassessed on day 28, gametocyte carriage was remeasured in Kuito at every scheduled visit, and a second blood sample for PCR genotyping was collected in the case of symptomatic parasitaemia after day 9 or at the end of follow-up in the presence of parasitaemia without symptoms. Rescue therapy (quinine hydrochloride 10 mg/kg/8 hourly for 7 days) was administered upon treatment failure.

2.4. Outcome classification

Children were withdrawn from the study in case of (i) vomiting any study dose twice, (ii) withdrawal of consent, (iii) onset of a serious febrile illness, (iv) intake of any drug with antimalarial properties, (v) missing any treatment dose, (vi) mixed parasitaemia or (vii) any protocol violation. Patients, who missed follow-up visits and did not come on successive days despite tracing, were considered lost to follow-up. Main outcomes were classified as follows (WHO, 2002): early treatment failure (ETF) — (i) progression to severe malaria by day 3, (ii) parasitaemia on day 2 > day 0, (iii) parasitaemia on day 3 $\geq$ 25% of day 0 or (iv) fever and parasitaemia on day 3; late clinical failure (LCF) — any time from day 4 to day 28 with (i) progression to severe malaria or (ii) fever plus parasitaemia; and late parasitological failure (LPF) — parasitaemia on day 28 without fever. All other children were classified as adequate clinical and parasitological responders (ACPR).

2.5. Laboratory methods

Capillary blood was obtained by fingerprick. Thick and thin films were prepared on the same slide and stained with 10% Giemsa (pH 7.2) for 15 min. Asexual parasitaemia was quantified against 200 to 500 leukocytes, assuming a white blood cell count of 8000/μl (WHO, 1991). Presence of gametocytes was recorded in Kuito. Ten per cent of the slides were reread randomly every day and any discordance was resolved by a third reader. External quality control on a random sample of slides

was carried out at the Ambroise Paré Hospital, Boulogne, France and showed concordance of more than 90%. Haemoglobin was measured using the Lovibond technique (Assistant Co., Sondheim Rhon, Germany).

Blood samples for PCR genotyping analysis were collected either on Isocode® kits (Schleicher & Schuell, Ecquevilly, France) or on Whatman no. 3 filter-paper and analysed at the Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium. DNA was purified as described previously (Irion et al., 1998) and a nested PCR was used for the analysis of two polymorphic genetic markers from *P. falciparum*: the three sequence families of the MSP1 block 2 repeat region and the two sequence families of the MSP2 repeat region. A recrudescent infection was defined as one that showed a match in size of at least one allele for both the MSP1 and MSP2 genes between the first and second samples. If any clone of a polyclonal primary infection was detected during a second episode, it was considered a recrudescence.

2.6. Sample size

Sample sizes were determined applying a Type I error risk of 0.05 and a projected 5% loss to follow-up. As no reliable estimates of antimalarial efficacy were available, all our calculations were based on an estimated 50% failure and a desired precision of 10%. This yielded a sample of about 95 children in each study group. Due to a lower than projected rate of enrolment and to logistic constraints, the sample size was limited to 80 patients per group in Caala.

2.7. Data entry and analysis

Records were entered and analysed on Epilnfo 6.04d (CDC, Atlanta, GA, USA). Each record was entered twice and data entry errors were checked. Baseline characteristics of patients were expressed either as percentages for categorical variables or as means with standard deviations for continuous variables. Parasitaemia at entry was computed as geometric mean parasitaemia with interquartile ranges. Anaemia was defined as haemoglobin (Hb) <11 g/dl (Brabin et al., 2001). Per cent gametocyte carriage was compared among study groups by χ^2 test. Therapeutic responses were expressed as the proportion of clinical failures (ETF + LCF) at day 14, non-corrected by PCR, and as the proportion of total failures (ETF + LCF + LPF) at day 28, corrected by PCR. Only recrudescence confirmed by PCR was considered as a true failure, whereas new infections and undetermined results were reclassified as ACPR.

2.8. Operational aspects

Team training and a 2 day pilot study were performed before the beginning of each study. Each study was co-ordinated by a field-based Medical Doctor (J.A., S.T.) present at each site throughout the study. The rest of the team was composed of two health promoters (screening), a clinical officer and a nurse (clinical examination and treatment, informed consent), three laboratory technicians and two tracers. Slides and records were kept in a locked room and given to the Angolan health authorities after the end of the study.

3. Results

A total of 2498 children were screened (813 in Caala and 1685 in Kuito; Figure 1). Of these, 619 met the inclusion criteria: 79 received CQ, 180 AQ, 173 SP, 97 AQ + AS and 90 SP + AS. One per cent (6/619) were lost to follow-up and 4.8% (30/619) were withdrawn. Reasons for withdrawal were intake of antimalarials (11), mixed parasitaemia (7), onset of serious illness (6), withdrawal of consent (3), vomiting the study drug twice (2) and protocol violation (1). Baseline characteristics were similar across treatment groups in each site, with the exception of sex distribution in Caala ($P = 0.01$) and in the ACT study in Kuito ($P = 0.04$) (Table 1).

PCR-corrected failure proportions at day 28 were very high in the CQ group (83.5% [66/79], 95% CI 74.1—90.5), high in the SP groups (Caala: 25.3% [20/79], 95% CI 16.7—35.8; Kuito: 38.8% [33/85], 95% CI 28.4—50.0), around 20% in the AQ groups (Caala: 17.3% [13/75], 95% CI 10.0—27.2; Kuito: 21.6% [21/97], 95% CI 14.3—30.6) and very low in the ACT groups (1.2% [1/84], 95% CI 0.0—6.4 in each combination AQ + AS and SP + AS) (Table 2).

Baseline gametocyte carriage in Kuito was 8.2% (7/85) for SP, 8.2% (8/97) for AQ, 20.2% (17/84) for AQ + AS and 25.0% (21/84) for SP + AS (Figure 2). While in the AQ, AQ + AS and SP + AS groups, the proportions decreased steadily after day 3 to reach <1% at the end of follow-up, the situation was different in the SP group where gametocyte carriage increased at each visit for 2 weeks, peaked at day 14 (64.5%) and then decreased during the following 2 weeks, although 28.8% still remained gametocytaemic at day 28.

Prevalence of anaemia significantly decreased between day 0 and day 28 in the two groups treated

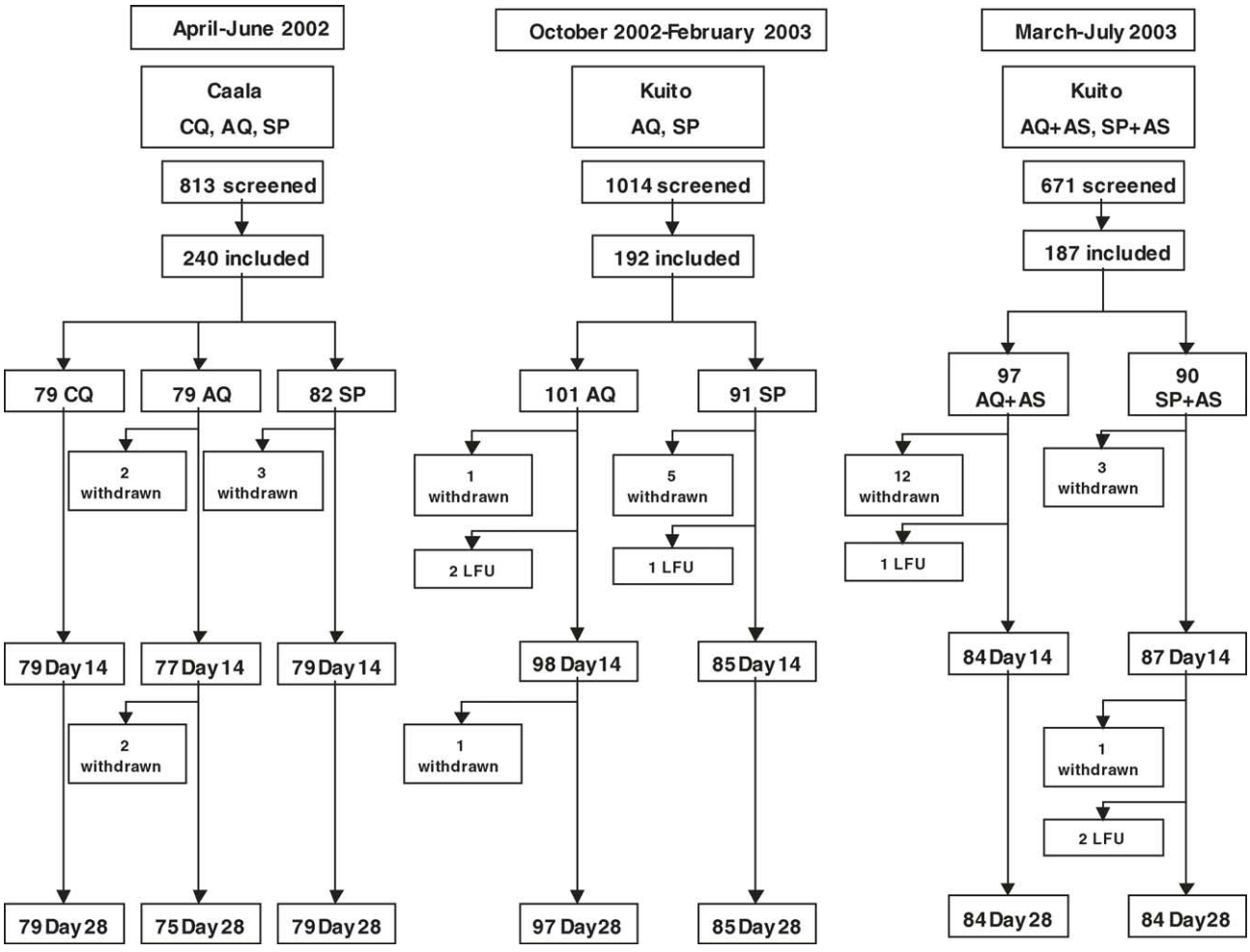

Figure 1 Details of study inclusion and follow-up (in vivo antimalarial efficacy studies, Caala and Kuito, Angola, 2002—2003).

with ACT: from 82.5% (80/97) to 39.3% (33/84) in the AQ + AS group ($P < 0.01$) and from 90.0% (81/90) to 36.9% (31/84) in the SP + AS group ($P < 0.01$). It was also the case, although less sharply, in both groups treated with SP: from 85.4% (70/82) to 66.0% (33/50) in Caala ($P < 0.01$), and from 93.4% (85/91) to 65.5% (36/55) in Kuito ($P = 0.01$), and in the group treated with AQ in Kuito, from 86.1% (87/101) to 44.8% (30/67) ($P = 0.01$).

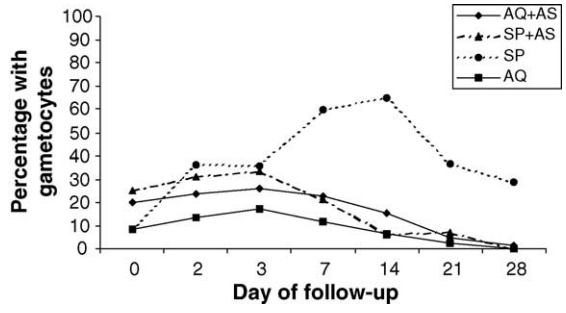

Figure 2 Percentages of patients with gametocytes at days 0, 2, 3, 7, 14, 21 and 28 according to drug group (in vivo antimalarial efficacy studies, Kuito, Angola, October 2002—July 2003).

4. Discussion

These in vivo studies provide important data on the efficacy of first- and second-line drugs in Angola when used alone or combined with an artemisinin derivative in a country where no recent information was available. It also shows the additional benefits of the use of ACT on gametocyte carriage and anaemia. Methodologically, it confirms the importance of extending follow-up to at least 28 days as studies with 14 days follow-up underestimate the true failure rates and are useful only in detecting high levels of drug resistance (Stepniewska et al., 2004; White, 2002), as shown here with CQ. In both

Table 1 Baseline (day 0) characteristics of included patients (in vivo antimalarial efficacy studies, Caala and Kuito, Angola, 2002—2003)

Characteristic	Caala			Kuito			
	CQ (*n* = 79)	AQ (*n* = 79)	SP (*n* = 82)	AQ(*n* = 101)	SP (*n* = 97)	AQ + AS (*n* = 97)	SP + AS (*n* = 90)
Number of males (%)	39 (49.4)	48 (60.8)	31 (37.8)	49 (48.5)	48 (52.7)	39 (40.2)	50 (55.6)
Age (months), mean (SD)	29.7 (13.7)	26.6 (15.1)	26.4 (13.7)	28.7 (15.0)	24.7 (15.3)	28.8 (14.7)	30.5 (14.4)
Axillary temperature (°C), mean (SD)	38.9 (1.00)	38.5 (1.08)	38.7 (1.18)	39.2 (0.83)	39.1 (1.09)	38.5 (1.3)	38.5 (1.2)
Weight (kg), mean (SD)	10.4 (2.4)	9.8 (2.2)	9.8 (2.4)	10.1 (2.5)	9.7 (2.4)	10.4 (2.5)	10.8 (2.6)
Asexual parasitaemia (/μl), geometric mean (IQR)	22 894 (11 647—43 032)	19 185 (9064—37 607)	22 749 (15 311—43 249)	27 272 (12 433—64 846)	24 344 (10 931—58 118)	19 117 (6868—61 813)	19 460 (9871—40 763)
Number (%) anaemic (Hb < ll g/dl)	619 (77.2)	59 (74.7)	70 (85.4)	86 (85.1)	85 (93.4)	80 (82.5)	81 (90.0)

Table 2 Failure proportions at day 14 (clinical criteria, no PCR adjustment) and at day 28 (clinical and parasitological failures, after PCR adjustment) (in vivo antimalarial efficacy studies, Caala and Kuito, Angola, 2002—2003)

Characteristic	Caala			Kuito			
	CQ (*n* = 79)	AQ (*n* = 75)[a]	SP (*n* = 79)	AQ (*n* = 97)[b]	SP (*n* = 84)	AQ + AS (*n* = 97)	SP + AS (*n* = 84)[c]
Clinical failure, day 14							
n	33	3	6	3	10	0	1
% (95% CI)	41.8(30.8—53.4)	3.9(1.0—10.2)	7.6(2.8—15.8)	3.1 (0.6—8.7)	11.7(5.8—20.6)		1.2(0—6.4)
Early treatment failure, *n* (%)	17(21.5)	2 (2.7)	5 (6.3)	2(2.1)	9(10.6)	0	1(1.2)
Late clinical failure, *n* (%)	37(46.8)	10(13.3)	11(13.9)	11(11.3)	15(17.6)	1 (1.2)	
Late parasitological failure, *n* (%)	12(15.2)	1(1.3)	4(5.1)	8 (8.2)	9(10.6)	0	0
Global failure, day 28							
n	66	13	20	21	33	1	1
% (95% CI)	83.5(74.1—90.5)	17.3(10.0—27.2)	25.3 (16.7—35.8)	21.6(14.3—35.8)	38.8 (28.4—50.0)	1.2(0—6.4)	1.2(0—6.4)

[a] *n* = 77 for the day 14 analysis, *n* = 75 for the day 28 analysis.
[b] *n* = 98 for the day 14 analysis, *n* = 97 for the day 28 analysis.
[c] *n* = 87 for the day 14 analysis, *n* = 84 for the day 28 analysis.

settings, a follow-up of only 14 days would have missed a large number of treatment failures. The data collected will certainly be of great value for the Angolan MOH and the changes of antimalarial drug policy that are planned.

The main limitation of these studies was the less than perfect randomization and the absence of allocation concealment. Randomization was limited to two arms in Caala, it was not done in the first study in Kuito, and it was started after 30 patients had already been recruited in the study on ACT. A proper comparison of study regimens is therefore difficult. A second limitation is that the exclusion of children with a previous history of treatment with a full dose of an antimalarial drug may have resulted in an underestimation of treatment failure as these patients probably harboured resistant parasites. Nevertheless, measures taken (such as the training of the staff or the use of qualified personnel to correctly ascertain the outcome) have probably limited potential biases. The validity of our data is further supported by the low percentage of losses to follow-up and of slide discordance.

Our results show that in vivo resistance to the first- (CQ) and second-line (SP) drugs have reached high levels in Caala and Kuito, confirming previous reports from East Africa (Bloland, 2001; Checchi et al., 2004; Stivanello et al., 2004). Resistance to CQ and SP is above the 25% value considered by WHO as the threshold for changing the drug policy (WHO, 2003). Efficacy of AQ is certainly higher than that of CQ and SP; however, it falls within the range (15—24%) suggested by WHO as the point when the process of looking for alternative therapy should start (WHO, 2003). This drug is therefore not a good alternative to CQ and SP.

In fact, as recommended by WHO, artemisinin-based combinations are the best option for replacing first-line regimens and should be implemented whenever possible (WHO/RBM, 2003). The advantages and other issues related to the use of such combinations have been well described (Bloland et al., 2003; Nosten and Brasseur, 2002). The question for us was which of CQ, AQ and SP was the best candidate to be combined with AS. Chloroquine was certainly not suitable, but AQ seemed a reasonable candidate. The efficacy of SP was certainly less than ideal. However, this drug is given as a single dose and this makes it a potential interesting partner drug for AS. This was our rationale for the evaluation of both AQ + AS and SP + AS in Kuito to provide the necessary data which would best assist the MOH in making a choice between these two and other possible ACTs.

The very low proportion of failures in both SP + AS and AQ + AS shows the tremendous benefit of adding an artemisinin derivative. Rather surprisingly, this benefit was also seen when adding AS to SP, despite high SP resistance. Both combinations represent a good alternative to be used as a first-line treatment in Caala and Kuito. Their price, when available in blister packs, is similar at about US$1.5 each. The SP + AS combination has the advantage that SP is administered as a single dose; however, considering the relatively high levels of resistance to this drug, the useful therapeutic lifespan of SP containing combinations may be rather short (D'Alessandro and Buttiëns, 2001). The combination of AQ + AS seems a better choice, not only because the efficacy of AQ alone is higher than that of SP alone, but also because a fixed combination should be marketed in the near future. These advantages may explain why some African countries such as Burundi, Liberia, Sudan and Sierra Leone have already adopted AQ + AS as the first-line therapy.

In summary, this series of studies provide solid information that will help the Angolan MOH to prepare a change in the current first-line antimalarial protocol. Our results in Caala and Kuito, although not applicable to the entire country, show that AQ + AS is the best option among the treatments we tested. Some authors may argue that the combination of AQ and SP could be a better choice, as suggested by a recent work in Uganda (Dorsey et al., 2002). However, considering the additional advantages of ACTs mentioned above (such as the potential to reduce transmission or to prevent the development of resistance), we believe that AS + AQ is a better alternative in Angola. However, the resistance to AQ monotherapy was around 20% and the combination with AS might not have a long useful therapeutic life, even if it was highly efficacious in this study. Policy decisions should be based on in vivo studies with a follow-up of at least 28 days and PCR genotyping to detect reinfections.

Conflicts of interest statement

The authors have no conflicts of interest concerning the work reported in this paper.

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