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Is amodiaquine failing in Rwanda? Efficacy of amodiaquine alone and combined with artesunate in children with uncomplicated malaria

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Summary We investigated the safety and efficacy of amodiaquine alone (AQ) and combined with artesunate (AQ + AS) in 308 Rwandan children 6–59 months old with uncomplicated *Plasmodium falciparum* malaria attending three sentinel sites. The two treatment regimes were well tolerated and no serious adverse events were recorded. After excluding new infections, children treated with AQ + AS had fewer clinical failures at day 28 after treatment than those treated with AQ alone: OR = 0.20 [95% CI: 0.06–0.57 ($P = 0.001$)]. Total (parasitological and clinical) failure was also significantly less frequent in the AQ + AS group: OR = 0.34 [95% CI: 0.17–0.67 ($P = 0.001$)]. When adjusting for study site, the hazard ratio for treatment failure was 0.37 [95% CI: 0.20–0.68 ($P = 0.001$)]. Combining AQ with AS increases the efficacy of the treatment but the apparent increase of AQ resistance observed in just a 1-year period is worrying and casts doubts on the suitability of implementing AQ + AS as first-line treatment in Rwanda. Alternative treatments should be identified and tested.

keywords amodiaquine, artesunate, uncomplicated malaria, Rwanda

Introduction

In Sub-Saharan Africa, *Plasmodium falciparum* chloroquine (CQ) resistance, first documented in eastern Africa in 1978, is now widespread and has required a change of the first-line antimalarial treatment to sulphadoxine–pyrimethamine (SP) by several countries (Shretta *et al.* 2000). In Africa, CQ resistance has resulted in increased malaria mortality (Trape 2001) and morbidity, e.g. transient clinical improvement and poor haematological recovery (Bloland *et al.* 1993). Furthermore, poor efficacy results increase drug costs for patients and the health system (Bloland *et al.* 1993).

The artemisinin derivatives cause a rapid and substantial decrease of the parasite load. However, when used alone and for <7 days, recrudescent infections are frequent because of the drug's short half-life (White & Olliaro 1998). Combination with a longer-acting drug such as mefloquine or amodiaquine (AQ) solves the problem of recrudescence, allows a shorter course of treatment and protects against the emergence of resistant strains because parasites are less likely to be resistant to both drugs (Hastings & D'Alessandro 2000).

In Rwanda, until recently, CQ and SP have been used as first- and second-line drugs for the treatment of uncomplicated malaria. However, *in vivo* tests carried out in 1999–2000 in four sentinel sites showed clinical failure (early and late) to CQ ranging from 16.7% to 56.1%, three of them over 50% (http://www.eanmat.org). Similarly, in 2000 SP clinical failure in three sites ranged between 11.6% and 44.7%. The need to rapidly deploy an efficacious and cheap alternative to CQ prompted Rwanda to choose in 2001 AQ + SP as the first-line treatment for uncomplicated malaria cases. A study in May–August 2001 (Rwagacondo *et al.* 2003) showed that AQ + SP was reasonably efficacious with a parasitological failure around 10%, a significantly better result than the other combination tested during the same study, SP + artesunate (AS). Nevertheless, AQ + SP has always been considered an interim strategy to be changed with a more efficacious combination, possibly with a long useful therapeutic life. As AQ associated with AS is a potential alternative to AQ + SP, we conducted in the same three sentinel sites a study comparing the efficacy of AQ alone and combined with AS in children with uncomplicated malaria, in order to obtain baseline data on these two treatments. The

first-line treatment AQ + SP was not evaluated because this had been carried out 1 year earlier in the same sites (Rwagacondo *et al.* 2003).

Materials and methods

Study site

The study was conducted in one urban/peri-urban health centre, Kicukiro near the capital town, and two rural health centres: Rukara towards the eastern border near the Kagera National Park and Mashesha, surrounded by rice fields, at 900 m a.s.l. Malaria is one of the major health problems in Rwanda and the first reason of attendance to health facilities (Ministère de la Santé 1999). Malaria transmission is not uniform, it is stable with seasonal peaks in the valleys and unstable on the hills with some districts at high risk of epidemics. The major vectors are *Anopheles gambiae* and *A. funestus*.

Patients

Children between 6 and 59 months of age with fever (axillary temperature ≥37.5 °C) and a presumptive diagnosis of clinical malaria were screened for malaria infection. Children weighing 5 kg or more and with a monoinfection with *P. falciparum* with a parasite density between 1000 and 100 000 parasites/μl were recruited if a parent or guardian gave informed consent. Children were excluded if they had (i) danger signs (unable to drink or breastfeed; vomiting more than twice in 24 h; recent history of convulsions; unconscious state or unable to sit or stand), (ii) signs of severe malaria, (iii) a packed cell volume (PCV) below 15%, (iv) clear history of adequate malaria treatment in the preceding 72 h, or (v) any evidence of chronic disease.

Study design

After final enrolment, the child's mother or guardian randomly selected from a box a slip of paper where treatment was specified, either a standard dose of AQ alone (10 mg/kg/day for 3 days) or AQ and AS (Dafra Pharma n.v., Belgium) (4 mg/kg/day for 3 days). All doses were given under direct supervision. Each child received paracetamol (10 mg/kg per dose) when needed and the parents were instructed to administer it when the child had fever. Children were observed for 1 h for vomiting and a replacement dose was given if necessary. Patients (parent/guardian) were asked to return to the clinic 24 and 48 h later for drug administration and for scheduled tests at 72 h, and at days 7, 14, 21, 28. If the patient did not report for scheduled visits every effort was made by the nurses to locate him/her at his/her home address. Parents were encouraged to return to the health centre any time the child was unwell. A blood slide for parasitaemia was collected at days 0, 3, 7, 14, 21 and 28. PCV was measured at days 0 and 14. Filter paper blood blots were collected for molecular biology on days 0, 14, 21 and 28 or on any day of recurrent parasitaemia after day 14. Children who experienced treatment failure were treated with a full course of quinine. The total sample size of 150 children per arm (distributed between the three sites) was calculated assuming a total failure (PCR corrected) of 14% for AQ alone (Rwagacondo *et al.* 2003) and 4% for AQ + AS. The Ministry of Health of Rwanda (MINISANTE) reviewed and approved the study.

Laboratory methods

Thick blood films were stained with Giemsa. Parasite density was determined on the basis of the number of parasites per 200 white blood cells (WBC) on a thick film assuming a total WBC count of 8000/μl. If gametocytes were seen, the gametocyte count was extended to 1000 WBC. PCV was measured by microhaematocrit centrifugation. If the child had a second episode of parasitaemia, blood samples on filter paper from the first and second episodes were used to type parasite strains. DNA was purified as described previously (Irion *et al.* 1998) and a nested PCR was adopted 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 matched in size 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.

Statistical methods

Data were double entered and validated using Epi Info 6.4b. Analyses were carried out with SPSS for windows (release 10.0.05) and STATA for Windows version 8.0 (Stata Corp., College Station, TX, USA, 2003). Outcomes were defined according to the new WHO classification: early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF), and adequate clinical and parasitological responses (ACPR) (WHO 2003). Clinical failure was defined as the sum of ETF and LCF, i.e. failure until day 3 after treatment included and parasitaemia of any density with fever between days 4 and 28. Total failure, besides clinical failure, includes reappearance of

parasitaemia after early disappearance, regardless of fever (ETF + LCF + LPF). Children were also considered treatment failures if they received rescue treatment on or before day 28 (Rwagacondo *et al.* 2003). In a secondary analysis, children were considered not to be parasitological or clinical failures if their parasitaemia between days 14 and 28 was classified as a new rather than recrudescent infection.

Two types of analysis were carried out: per protocol and survival analysis. The per protocol analysis is the classical method for calculating drug efficacy. In this approach, only patients with known efficacy endpoints are retained for analysis, while the others are excluded and do not contribute to the denominator. The survival analysis is the new method recommended by WHO to estimate drug efficacy. The analysis was carried out on all failures (ETF + LCF + LPF). Its benefits include the ability of using data from patients withdrawn or lost during follow-up, the calculation of mean time to failure and of a reasonably unbiased estimate of failure rates (WHO 2003).

For the per protocol, the chi-squared test was used to compare the proportion of clinical and total failures between the two groups and the 95% CI were computed for the corresponding crude odds ratio (OR). Mantel Haenzel (MH) site-specific ORs for failures were computed as well as the adjusted MH ORs (after performing test of homogeneity). For the risk of total failure at day 28 corrected by PCR, a multivariate analysis using logistic regression was used to analyse the effect of other potential risk factors for treatment failures (age, anaemia, site).

For the survival analysis, the time of participation, corresponding to the number of days between day 0 and the final event, was calculated for each patient. The final event corresponded either to an outcome according to the new WHO classification (ETF, LCF, LPF and ACPR) or, in case a patient did not complete the follow-up, to the day of the last visit the patient was seen. Kaplan–Meier survival estimates were computed. The cumulative failure risks were calculated with the formula: 1 − (survival function) and compared with a Cox regression adjusting for potential confounding factors in order to obtain an adjusted HR. The proportional hazard ratio (HR) assumption was tested using the Schoenfeld test in STATA.

Results

A total of 308 children satisfying the entry criteria were recruited between February and April 2002, 91 (29.5%) from Kicukiro, 122 (39.6%) from Mashesha and 95 (30.8%) from Rukara. Baseline characteristics of the two study groups are shown in Table 1. Two patients in the AQ-alone group and one in the AQ + AS group were lost to follow-up before day 14; three additional patients in the AQ + AS group were lost to follow-up between days 15 and 28 (Figure 1). Both treatment regimens were well tolerated and no serious adverse events were recorded.

No ETF was observed. Three LCF were observed at day 14, two of 148 (1.4%) in the AQ alone group and one of 157 (0.6%) in the AQ + AS group. By day 28, there were 35 LCF, 22 in the AQ (14.9%) group and 13 (8.4%) in the AQ + AS group (Table 2). The odds for clinical failure were higher for children treated with AQ alone than in those treated with AQ + AS (OR = 0.53; 95% CI: 0.24–1.15; $P = 0.1$) but the difference was not statistically significant. Most of the infections (33/35) in the LCF cases could be genotyped. Those we were unable to genotype were considered as recrudescence. New infections were more frequent in the AQ + AS (8/13, 61%) than in the AQ group (1/22, 4.0%) and, when excluded, the risk of LCF was significantly lower in the AQ + AS group (OR = 0.20, 95% CI: 0.06–0.57; $P = 0.001$).

Five children were parasitaemic at day 14 irrespective of fever, three of 148 (2.0%) in the AQ alone and two of 157 (1.3%) in the AQ + AS group. By day 28, 75 children were parasitaemic, 47 (31.8%) in the AQ group and 28 (18.2%) in the AQ + AS group (Table 2). Overall, AQ + AS was significantly more effective than AQ alone: crude OR = 0.48 (95% CI: 0.27–0.84; $P = 0.008$) [MH OR

Table 1 Baseline characteristics of patients at enrolment

	AQ alone ($n = 150$)	AQ + AS ($n = 158$)
Mean age in months (SD)	29.3 (16.1)	26.3 (14.4)
Mean weight in kg (range)	11.6 (6.2–21.0)	11.5 (5.7–20.0)
Antimalarial drug taken before inclusion, *n* (%)	5 (3.3)	11 (6.1)
Mean temperature in °C (SD)	38.4 (0.82)	38.5 (0.83)
Parasite density per µl, geometric mean (range)	14 040 (1040–99 320)	20 522 (1000–99 000)
Gametocyte rate, *n* (%)	1 (0.6)	0 (0.0)
Mean PCV (SD)	30.8 (5.1)	30.2 (5.1)

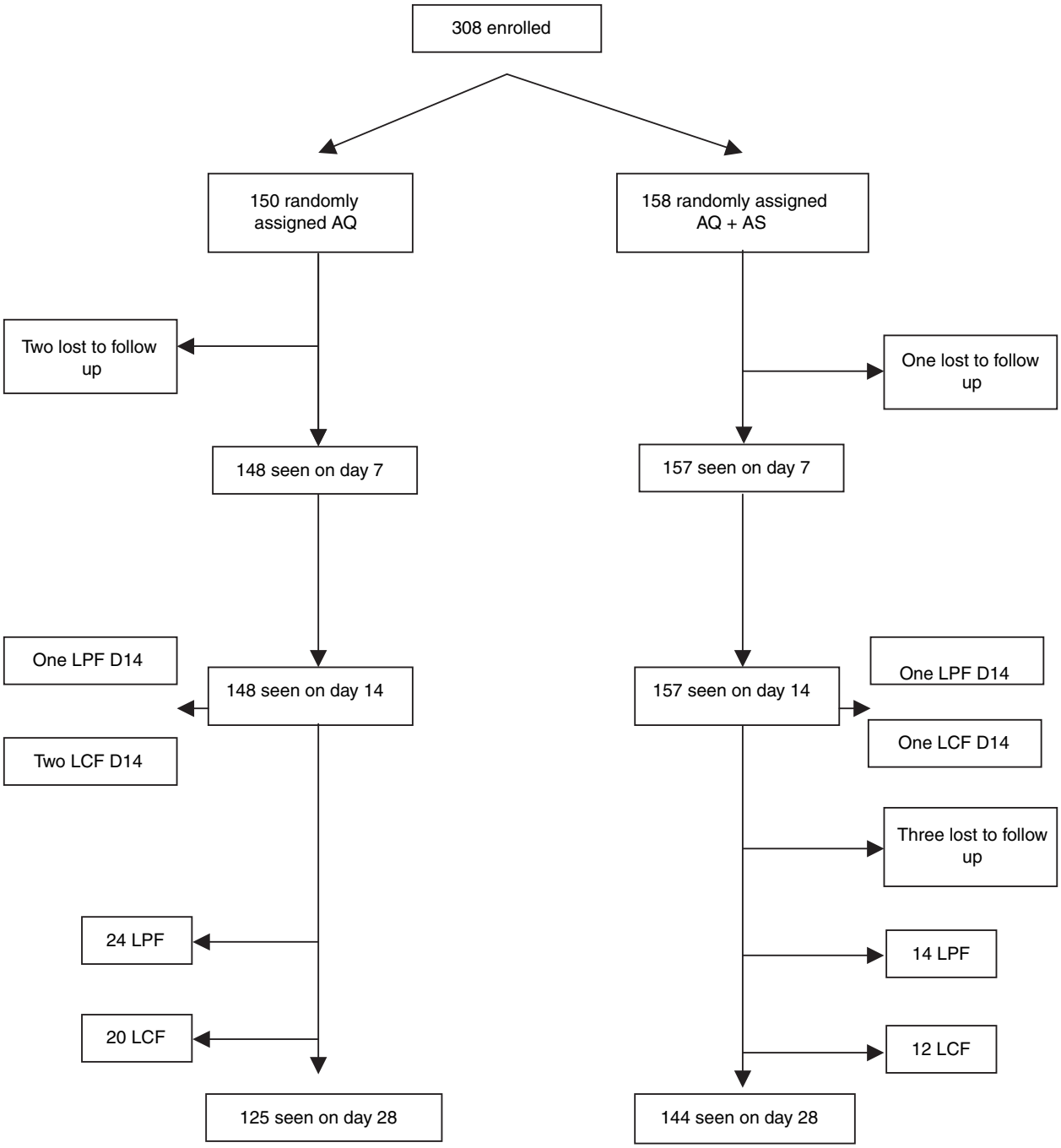

Figure 1 Trial profile.

adjusted for site OR = 0.47 (95% CI: 0.27–0.81; $P = 0.006$); chi-square test of homogeneity, $P = 0.15$]. Site-specific ORs for failures (AQ + AS *vs.* AQ) were significant in the urban/peri-urban site of Kicukiro and in Mashesha, 0.27 ($P = 0.014$) and 0.33 ($P = 0.023$) respectively, while in Rukara the risk of total failures was similar in the two groups (OR = 0.94; $P = 0.88$). After excluding new infections, AQ + AS was more efficacious

Table 2 Total failure (LCF + LPF) at days 14 and 28 and clinical failure (LCF) between days 14 and 28, by group and by sentinel site

	AQ alone	AQ + AS	*P* value
LCF at day 14 (%)	*n* = 148	*n* = 157	
	2 (1.4)	1 (0.6)	0.61
LCF at day 28 (%)	*n* = 148	*n* = 154	
Kicukiro	3/43 (7.0)	1/47 (2.1)	0.34
Mashesha	9/61 (14.8)	5/61 (8.2)	0.39
Rukara	10/44 (22.7)	7/46 (15.2)	0.42
Total	22 (14.9)	13 (8.4)	0.1
LCF at day 28, PCR corrected*			
Kicukiro	3/43 (7.0)	0/47 (0.0)	0.1
Mashesha	8/61 (13.1)	2/61 (3.3)	0.09
Rukara	10/44 (22.7)	3/46 (6.5)	0.03
Total	21 (14.2)	5 (3.2)	0.001
Total failure at day 14 (%)	*n* = 148	*n* = 157	
	3 (2.0)	2 (1.3)	0.67
Total failure at day 28 (%)	*n* = 148	*n* = 154	
Kicukiro	15/43 (34.9)	6/47 (12.8)	0.02
Mashesha	17/61 (27.9)	7/61 (11.5)	0.03
Rukara	15/44 (34.1)	15/46 (32.6)	1.0
Total	47 (31.8)	28 (18.2)	0.008
Total failure at day 28 (%) PCR corrected*			
Kicukiro	12/43 (27.9)	2/47 (4.3)	0.003
Mashesha	12/61 (19.7)	4/61 (6.6)	0.05
Rukara	12/44 (27.3)	9/46 (19.6)	0.45
Total	36 (24.3)	15 (9.7)	0.001

*Only new infections excluded.

overall [crude OR = 0.34 (95% CI: 0.17–0.67; $P < 0.001$)], and in Kicukiro and in Mashesha: 0.11($P = 0.002$), 0.27 ($P = 0.03$) respectively, while in Rukara the risk was still not significantly different between treatment groups (OR = 0.65; $P = 0.39$). The multivariate risk factor analysis showed that site was a significant risk factor for failure after adjusting for the effect of treatment (AQ + AS *vs.* AQ): compared with Mashesha, the risk of failure was two times higher in Rukara (OR = 2.10; 95% CI: 1.01–4.38) and there was a trend for a higher risk in Kicukiro (OR = 1.26; 0.57–2.77) without reaching significance. The adjusted OR for failure (AQ + AS *vs.* AQ) was 0.33 (95% CI: 0.17–0.63; $P = 0.001$).

The Kaplan–Meier cumulative risk at day 28 for all failures was 32% [95% CI: 25–40] for the AQ-alone group and 19% (95% CI: 13–26) for the AQ + AS group. When excluding new infections, this risk was 25% (95% CI: 19–33) for the AQ-alone group and 11% (95% CI: 7–17) for the AQ + AS group (Figure 2). Using the Cox regression, when adjusting for study site, AQ + AS was significantly more efficacious than AQ alone, HR: 0.37 (95% CI: 0.20–0.68; $P = 0.001$) (test of proportional HR,

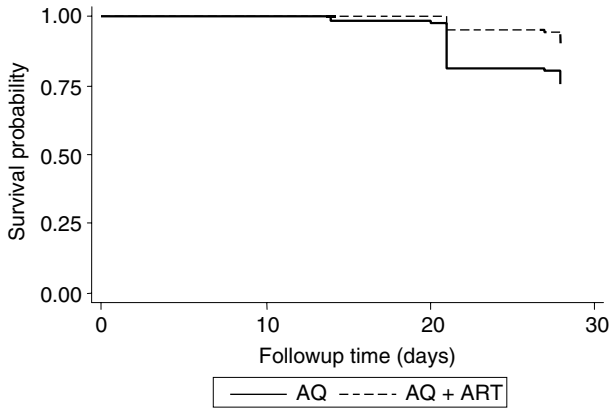

Figure 2 Kaplan–Meier survival estimates (PCR corrected), by treatment.

$P = 0.13$). The risk of treatment failure, when adjusting for treatment, was two times higher in Rukara compared with Mashesha (HR: 1.94, 95% CI: 1.01–3.72) while it was not significantly different between Kicukiro and Mashesha (HR: 1.20, 95% CI: 058–2.46).

Gametocyte rates were low (the highest value was 7% at day 3 for the AQ + AS group) and not significantly different between the two treatment groups.

The PCV was measured in all children at day 0 and in 304 children at day 14. The PCV changes between days 0 and 14 were not significantly different between the two groups (data not shown).

Discussion

In this study the combination AQ + AS was significantly more efficacious than AQ alone in treating uncomplicated malaria. Nevertheless, it should be noticed that there were important differences between sites and according to the outcome considered. The percentage of total failures was significantly lower for AQ + AS in Kicukiro and Mashesha but not in Rukara. When considering clinical failure, the two treatments were not significantly different in Kicukiro and Mashesha while in Rukara, when excluding new infections, the percentage of failures was significantly lower for AQ + AS. It is also interesting to notice that most of the infections in the AQ group were classified as recrudescence while more than half of those in the AQ + AS group were classified as new infections. Obviously this does not mean that children treated with AQ + AS had a different and higher risk of being newly infected, such risk was evenly distributed between the two study groups. However, in children treated with AQ alone, several malaria infections were not cleared and were classified as recrudescence, even if new infections were acquired during the follow-up period. Therefore, the 3-day course of AS can considerably improve the clinical outcome when associated with another drug with a longer half-life. However, the partner drug has to be efficacious as well, otherwise the infection will not be completely cleared or, in places where transmission is intense, new infections will appear during the period when the partner drug should still be active. Paradoxically, the observed difference in efficacy between a given drug used alone and in combination with AS will be higher when the resistance to the monotherapy is high (Von Seidlein *et al.* 2000; Adjuik *et al.* 2002; Gil *et al.*) and this might give the false impression that the combination could be considered as a possible first-line treatment. Indeed, in Rukara, adding AS to AQ decreased considerably the clinical failure, from more than 20% to about 6%, although the total failure was not significantly different between the two treatment regimens. The question is how long an artemisinin-containing combination will remain efficacious when the partner drug is failing. The combination AS–mefloquine has been adopted by some Asian countries as first-line treatment against falciparum malaria, a near-desperate therapeutic choice in front of multidrug resistance (Wongsrichanalai *et al.* 2000). Its use has halted the progression of mefloquine resistance and reduced the *P. falciparum* malaria incidence, possibly because of its anti-gametocyte properties (Nosten *et al.* 2000). However, transmission intensity in South-ast Asia is much lower than that found in many parts of Africa and the probability of new infections emerging within 4 weeks after treatment is small. This means that even if the two components of the combination have a very different elimination half-life, such as is the case for AS-mefloquine, it is unlikely that a new infection will be exposed to suboptimal and selective levels of the drug with the longer elimination. In areas of high transmission this is obviously possible and new infections able to survive to suboptimal level of the drug combined to AS will appear as soon as the latter has been eliminated. Probably the period between treatment and emergence will become progressively shorter as the tolerance of the parasites to the drug will increase. This is illustrated in our study by the substantial number of new infections observed among children treated with AQ + AS. Strictly speaking these infections are excluded when estimating treatment efficacy. However, it should be considered that they have emerged at a time when AQ should have suppressed them if it was still efficacious. It is difficult to know what their evolution beyond the follow-up period would have been but it is likely that part or most of them would have resulted into a clinical attack (Dorsey *et al.* 2002) requiring a new course of antimalarial treatment a few weeks after the first one. Besides the deleterious effects on the patient's health and the cost for the health system, parasites exposed to decreasing drug level, even if new infections, would become increasingly tolerant to the drug, accelerating the spread of resistance. The logical consequence is that, wherever possible, artemisinin derivatives should be combined with less widely used drugs than AQ or with truly novel drugs such as piperaquine (Bloland 2003).

In two sites, Kicukiro and Rukara, we found a higher resistance to AQ alone than just 1 year earlier (Rwagacondo *et al.* 2003). In 2001 total failure to AQ alone was 14.3% in Kicukiro and 25.8% in Rukara. Even when corrected by PCR analysis these figures (still 14.5% for Kicukiro and 19.4% for Rukara) are considerably lower than those reported here. It is difficult to estimate whether this is a chance finding or a true increase in resistance, but these results are worrying considering also that neighbouring Burundi has just opted for the combination AQ + AS as first-line treatment. It has been reported that AQ is effective for treating uncomplicated falciparum malaria in areas of West and Central Africa where CQ resistance is prevalent (Brasseur *et al.* 1999).

However, several recent studies have reported a less-than-optimal efficacy of AQ (Adjuik *et al.* 2002; Checchi *et al.* 2002), even where its use is low (Schellenberg *et al.* 2002). Although AQ could be still efficacious where CQ resistance is moderate, it might fail where CQ resistance is widespread (Ringwald *et al.* 2000) and where its extensive use as first-line antimalarial treatment would increase the drug pressure and the selection of resistant parasites. This might explain the apparent rapid increase of AQ resistance we observed as CQ clinical failure in Rukara in 1999–2000 was estimated at over 50% (http://www.eanmat.org), a figure that prompted the Ministry of Health to change its antimalarial drug policy from CQ to the association AQ + SP.

The percentage of failures at day 14 was extremely low and, had the patients not been followed until day 28, AQ resistance would have been greatly underestimated. The appropriate period of follow-up depends on the drug clearance, six times the elimination half-life, and it is defined at 28 days for AQ, CQ and SP (WHO 2003). Although a 28-day follow-up has substantial logistic and cost implications, not least the need of performing molecular analysis to distinguish between a recrudescence and a new infection, it has the advantage of estimating more precisely and at an earlier stage the magnitude of drug resistance. Indeed, finding moderate resistance to CQ, AQ or SP 14 days after treatment is already an indication that the drug is badly failing and that it should not be used for treatment. A 28-day follow-up would identify unacceptable drug resistance much earlier and would allow more time for a possible change of the drug policy.

We used survival analysis, the method newly recommended by WHO, to estimate drug efficacy (WHO 2003). It has the advantage of considering patients who have been withdrawn or lost to follow-up and it should give a reasonably unbiased estimate of the failure rates. The figures obtained with the per protocol and survival analysis, not surprisingly, are extremely similar as the percentage of patients lost to follow-up or withdrawn was extremely low. Therefore, the two methods will give similar results when the number of patients lost to follow-up or withdrawn is low.

The current first-line treatment in Rwanda is the combination AQ + SP, implemented in 2001 and considered as an interim strategy. The most recent data collected in 2001 show reasonable efficacy (PCR corrected): total failure was around 13%. However, this is a summary estimate and large variations in the different study sites were found: 13% in Kicukiro, 4% in Mashesha and 27% in Rukara. One year later, AQ resistance seems to have increased and AQ + SP efficacy is likely to have declined further. Therefore, this drug policy should be re-considered and alternative treatments should be identified. Although we observed an improvement in treatment efficacy when combining AQ with AS, the apparent increase of AQ resistance observed in just 1 year in two of three sites is worrying and casts doubt on the suitability of implementing it as first-line treatment in Rwanda.

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