

Efficacy and safety of re-treatment with the same artemisinin-based combination treatment (ACT) compared with an alternative ACT and quinine plus clindamycin after failure of first-line recommended ACT (QUINACT): a bicentre, open-label, phase 3, randomised controlled trial

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Summary

Background Quinine or alternative artemisinin-based combination treatment (ACT) is the recommended rescue treatment for uncomplicated malaria. However, patients are often re-treated with the same ACT though it is unclear whether this is the most suitable approach. We assessed the efficacy and safety of re-treating malaria patients with uncomplicated failures with the same ACT used for the primary episode, compared with other rescue treatments.

Methods This was a bicentre, open-label, randomised, three-arm phase 3 trial done in Lisungi health centre in DR Congo, and Kazo health centre in Uganda in 2012–14. Children aged 12–60 months with recurrent malaria infection after treatment with the first-line ACT were randomly assigned to either re-treatment with the same first-line ACT, an alternative ACT, which were given for 3 days, or quinine-clindamycin (QnC), which was given for 5–7 days, following a 2:2:1 ratio. Randomisation was done by computer-generated randomisation list in a block design by country. The three treatment groups were assumed to have equivalent efficacy above 90%. Both the research team and parents or guardians were aware of treatment allocation. The primary outcome was the proportion of patients with an adequate clinical and parasitological response (ACPR) at day 28, in the per-protocol population. This trial was registered under the numbers NCT01374581 in ClinicalTrials.gov and PACTR201203000351114 in the Pan African Clinical Trials Registry.

Findings From May 22, 2012, to Jan 31, 2014, 571 children were included in the trial. 240 children were randomly assigned to the re-treatment ACT group, 233 to the alternative ACT group, and 98 to the QnC group. 500 children were assessed for the primary outcome. 71 others were not included because they did not complete the follow-up or PCR genotyping result was not conclusive. The ACPR response was similar in the three groups: 91.4% (95% CI 87.5–95.2) for the re-treatment ACT, 91.3% (95% CI 87.4–95.1) for the alternative ACT, and 89.5% (95% CI 83.0–96.0) for QnC. The estimates for rates of malaria recrudescence in the three treatment groups were similar (log-rank test: $\chi^2=0.22$, $p=0.894$). Artemether-lumefantrine was better tolerated than QnC ($p=0.0005$) and artesunate-amodiaquine ($p<0.0001$) in the modified intention-to-treat analysis. No serious adverse events were observed. The most common adverse events reported in the re-treatment ACT group were anorexia (31 [13%] of 240 patients), asthenia (20 [8%]), coughing (16 [7%]), abnormal behaviour (13 [5%]), and diarrhoea (12 [5%]). Anorexia (13 [6%] of 233 patients) was the most frequently reported adverse event in the alternative ACT group. The most commonly reported adverse events in the QnC group were anorexia (12 [12%] of 98 patients), abnormal behaviour (6 [6%]), asthenia (6 [6%]), and pruritus (5 [5%]).

Interpretation Re-treatment with the same ACT shows similar efficacy as recommended rescue treatments and could be considered for rescue treatment for *Plasmodium falciparum* malaria. However, the effect of this approach on the selection of resistant strains should be monitored to ensure that re-treatment with the same ACT does not contribute to *P falciparum* resistance.

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Introduction

Since the early 2000s, a downward trend in the malaria burden has been observed, resulting in reductions of 37%

in malaria incidence and 60% in malaria mortality worldwide. Nevertheless, the malaria burden remains substantial, with an estimated 214 million cases in 2015,

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Research in context

Evidence before this study

Artemisinin-based combination therapies (ACTs) are recommended for first-line treatment of uncomplicated *Plasmodium falciparum* malaria. If recurrent malaria occurs before day 28, WHO recommends an alternative ACT in addition to quinine accompanied by an antibiotic having antiparasmodial properties (such as clindamycin, tetracycline, or doxycycline). This recommendation is based on expert opinion. In real-life situations, the prescriber is not always aware of former treatments and patients might be re-treated with the first-line ACT. We searched in PubMed for randomised trials without date or language restrictions, using the MeSH terms “uncomplicated malaria”, “retreatment”, “rescue therapy”, or “second-line treatment”. We did not identify any trial that had investigated the efficacy and safety of re-treatment with the first-line ACT nor with an alternative ACT. Therefore, we undertook this trial to provide evidence. Two malaria-endemic countries using different first-line ACTs were selected to host

the trial (artesunate-amodiaquine in the DR Congo and artemether-lumefantrine in Uganda).

Added value of this study

Most ACT treatment failures are reinfections. Our findings show that the treatment of recurrent malaria episode with first-line recommended ACT has a similar efficacy as during the initial first-line treatment and as the recommended rescue treatments. Furthermore, we provide evidence to confirm WHO experts' opinion recommending an alternative ACT as rescue treatment in addition to quinine.

Implications of all the available evidence

Most primary and community health centres in Africa have only one ACT available. The use of the same ACT as a rescue treatment might be considered. However, correct dosing strategies should be implemented and the effect of re-treatment on the population genetics of circulating *P falciparum* strains needs to be monitored.

and 438 000 related deaths.¹ Within this context, the availability of effective treatments remains essential for sustaining these achievements.

Over a decade ago, WHO recommended artemisinin-based combination therapies (ACTs) for the treatment of uncomplicated malaria, in response to the spread of resistance to commonly used antimalarial drugs, namely chloroquine and sulfadoxine-pyrimethamine. Combining two drugs with different mechanisms of action aimed to reduce the emergence of parasitic resistance.² All malaria-endemic countries have adopted ACT as first-line treatment for uncomplicated malaria. Both DR Congo and Uganda have high malaria incidence. A child younger than 5 years can have up to ten malaria episodes during a year.^{3,4} Artemether-lumefantrine (AL) is the recommended first-line treatment in Uganda, but AL and artesunate-amodiaquine (ASAQ) are both recommended in DR Congo, with ASAQ being the most widely used. Quinine is recommended for any treatment failure, as a rescue treatment.

Quinine has a short half-life, requiring repeated dosing over 7 days. Treatment with quinine can lead to several adverse events and poor compliance.⁵⁻⁷ Quinine can be less effective than ACT in patients with uncomplicated malaria.^{5,8} Since most of the treatment failures in patients given AL or ASAQ seem to be for new infections with low parasite densities,^{9,10} it might be possible to re-treat patients with the same ACT used for the primary episode. However, there is little evidence on the efficacy and safety of recommended rescue treatments.¹¹ We therefore aimed to investigate ACT re-treatment in children who had been treated for uncomplicated malaria, and who were experiencing a recurrent infection by assessing the efficacy and safety of re-treatment with the same ACT used for the primary

episode compared with that of an alternative ACT or quinine and clindamycin (QnC).

Methods

Study design

This study was a bi-centre, phase 3b, randomised, open-label, three-arm trial done at Lisungi Health Center in Kinshasa, DR Congo, and Kazo Health Center in Mbarara, Uganda. Ethical approval was obtained from the Ethics Committee of the School of Public Health at the University of Kinshasa (DR Congo), the Uganda National Council for Science and Technology (Uganda), and the Ethics Committee of the University of Antwerp (Belgium). This trial was done in accordance with the Good Clinical and Laboratory Practices, applicable regulatory requirements, and the Declaration of Helsinki. The trial protocol is available online.

Participants

Children aged 12–59 months, weighing at least 9 kg, and attending study sites for fever or history of fever were given first-line ACT (ASAQ in DR Congo and AL in Uganda) and passively followed up (malaria was screened only in presence of symptoms) for 42 days (the pre-trial phase). The treatment was directly observed by a study nurse. The 42-day PCR-adjusted efficacy was 93·5% (data not shown) for AL in Uganda and 92·8% for ASAQ in DR Congo.¹² Microscopy was used to diagnose malaria in children, who were then assessed for eligibility in the randomised trial. To be included in the trial, children had to fulfil all the following criteria: recurrent *Plasmodium falciparum* infection with fever or history of fever, willingness and ability of the parents or guardians to comply with the study protocol for the duration of the study, and written informed consent from parents or

For the trial protocol see
[http://www.trialsjournal.com/
content/14/1/307](http://www.trialsjournal.com/content/14/1/307)

guardians. Exclusion criteria were: known hypersensitivity to or previous serious drug-related adverse events with the study drugs, severe malaria or danger signs, treatment failure within 14 days after treatment of the primary episode; and bodyweight of less than 9 kg. Two written informed consents were obtained, the first before treatment with the first-line ACT (pre-trial phase) and the second before recruitment in the randomised trial.

Randomisation and masking

Patients were randomly assigned to receive either the same ACT treatment as the first-line treatment (re-treatment ACT group), an alternative ACT to the first-line treatment (alternative ACT group), or QnC (QnC group), in a ratio of 2:2:1. In DR Congo, the re-treatment ACT group were treated with ASAQ and the alternative ACT group were treated with AL, and in Uganda the re-treatment ACT group were treated with AL and the alternative ACT group received ASAQ. Patients assigned to the QnC group received the same QnC treatment in both countries. Treatment was randomly allocated in blocks by country according to a randomisation list that was automatically generated by the statistical team at the University of Antwerp before starting the study. The treatment allocations were sealed in envelopes that were opened by the study clinician immediately after obtaining the written informed consent. This was an open-label study, therefore, investigators and participants (or their parents or guardians) were aware of study treatment

Procedures

Children received ASAQ, AL, or QnC and were followed up for 28 days. Parents or guardians were asked to return to the clinic for follow-up at scheduled visits on days 1, 2, 3, 7, 14, 21, and 28. They were also encouraged to attend the clinic any time the child was unwell.

Study medications were quality assured. AL (Novartis, Basel, Switzerland) was administered twice a day, orally for three days. Tablets containing 20 mg of artemether and 120 mg of lumefantrine were administered with a cup of milk (to improve lumefantrine absorption). Dosage was according to manufacturer's instructions: one tablet for children weighing 5–14.9 kg, two tablets for children weighing 15–24.9 kg, and three tablets for children weighing 25–34.9 kg. ASAQ (Sanofi, Casablanca, Morocco) was administered once a day, orally for 3 days. Tablets contained 50 mg of artesunate and 135 mg of amodiaquine for children weighing 9–17.9 kg; 100 mg of artesunate and 270 mg of amodiaquine for children weighing 18–35.9 kg. Both ACTs were co-formulated. Quinine (Sanofi, Gentilly, France) was administered three times a day, orally for 7 days. Tablets of 125 mg were available. Half a tablet was given to children weighing 9–11.9 kg, one tablet to those weighing 12–19.9 kg, one and a half tablets to those weighing 20–27.9 kg, and two tablets to those weighing 28–35.5 kg. Clindamycin (Pfizer SA/NV Brussels, Belgium) granules were

suspended in a 75 mg/5 mL solution (in sucrose, ethyl parahydroxybenzoate, poloxamer, and artificial cherry oil), which was administered orally twice a day for at least 5 days at a dosage of 10 mg/kg.

All ASAQ and AL treatments were administered on site, under the direct supervision of study nurses. For logistical reasons, in DR Congo, children assigned to the QnC group were admitted to hospital for the first 3 days of the trial. On the fourth day, the first two doses were given under supervision, and the third, evening dose was given at home by the parent or guardian. After the drug was administered on site, children were kept at the clinic for 60 min. A full dose was repeated if vomiting occurred within 30 min of administration, and a half dose was administered if vomiting occurred between 30 and 60 min. In case of persistent vomiting, the patient was withdrawn from the study and referred to the health facility for parenteral treatment.

Microscopy examination involved collecting thick and thin blood films at each visit and staining with 10% Giemsa within 10 min. Parasites were counted over 200 white blood cells, and density per μL estimated assuming 8000 white blood cells per μL . Each slide was read independently by two microscopists; a third reading was done in case of discrepancies between the first two readings. The Institute of Tropical Medicine in Antwerp was responsible for microscopy external quality control and two laboratory-specific monitoring visits were done at both sites by experts from the malaria unit. Haemoglobin was measured at days 0, 14, and 28, by use of a portable spectrophotometer (Hemocontrol, EKF Diagnostics, Barleben, Germany). At each visit, dried blood spots were obtained on filter paper (Whatman 3 MM, Maidstone, UK) and air-dried before storage. DNA was extracted from dried blood spots according to the manufacturer's instructions (QIAamp DNA Mini Kit, Hilden, Germany). To distinguish between a new infection and a recrudescence, we did PCR genotyping of the polymorphic regions of the gene encoding merozoite surface protein-1 (*MSP1*), the gene encoding merozoite surface protein-2 (*MSP2*), and glutamate-rich protein (*GLURP*), as described previously.^{13,14} Briefly, we compared the genetic signatures of the samples at day 0 (D0) and day X (DX, at treatment failure). If at least one identical allele was found in these paired samples at each of the three loci, the failure was classified as recrudescence. When the amplification reaction for one locus proved to be negative for either one or both of the paired samples, the outcome was classed as indeterminate. To assess genotyping-adjusted outcomes, recurrent parasitaemia due to new infections was considered as adequate clinical and parasitological response (ACPR).

Outcome

The primary outcome was the proportion of children with ACPR after PCR adjustment at day 28 and measured in the per-protocol population. The ACPR was defined as

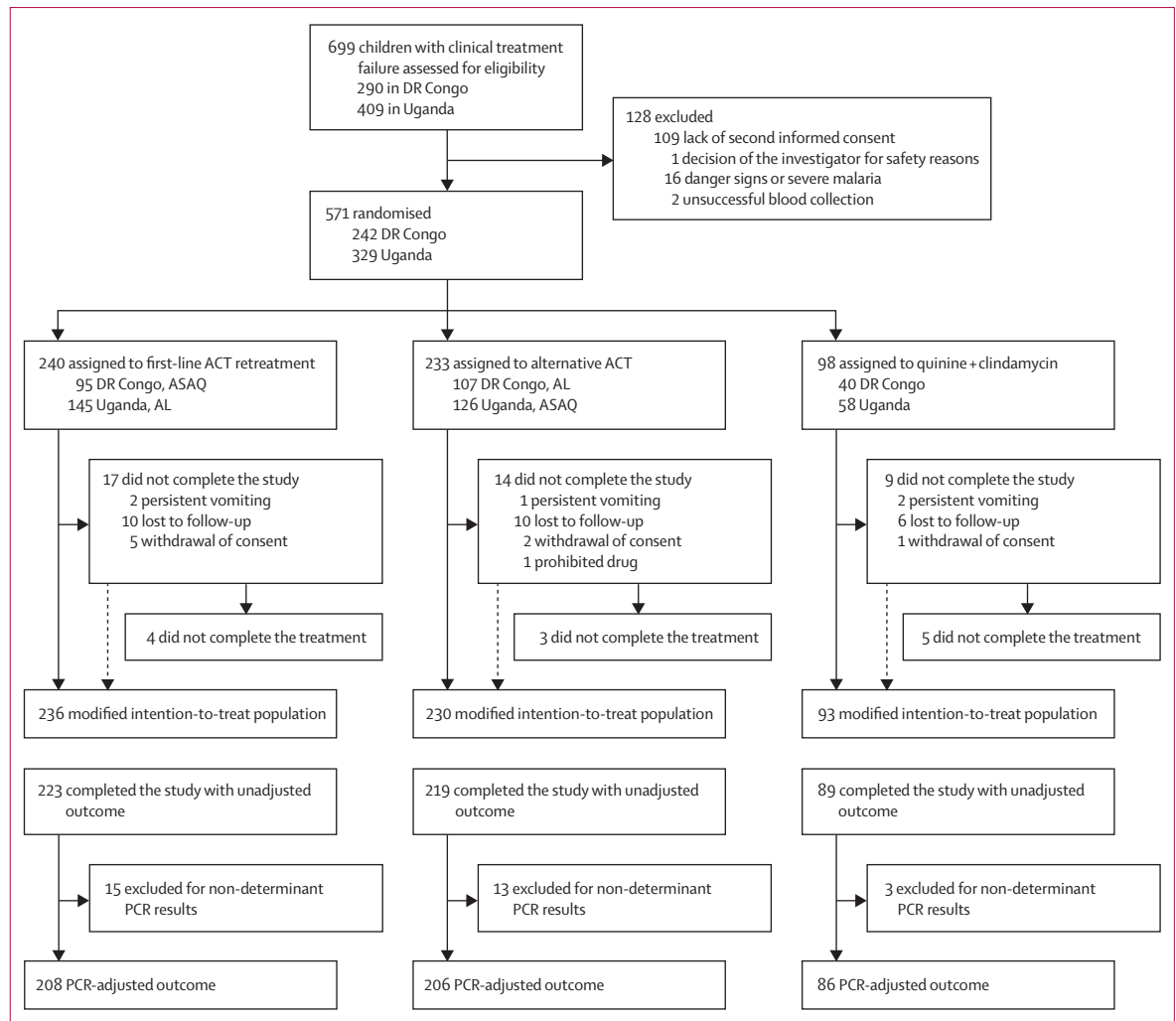


Figure 1: Trial profile for 28 days of follow-up after rescue treatment in QUINACT study participants at the Lisungi Health Centre (DR Congo) and the Kazo Health Centre (Uganda), 2012–14

the absence of parasitaemia at the end of the study follow-up period (day 28), regardless of tympanic temperature, without having previously met any of the criteria for early and late treatment failure.¹⁵ In the PCR-adjusted analyses, children with recurrent infection were considered ACPR if this was classified as a new infection. Primary outcome was assessed in children who completed 28 days of follow-up without having recurrent malaria and those who experienced recurrent malaria but in whom PCR genotyping revealed a new infection (ie, initial parasites had been successfully cleared before the onset of a new episode), with children who had indeterminate PCR genotyping results being excluded from the data analyses.

Secondary efficacy outcomes were assessed in the per-protocol population. This population included children who had a known efficacy endpoint, without any criteria of protocol violation. They included fever clearance time (FCT), defined as the time, in days, from randomisation

to the first two consecutive measurements of tympanic temperature lower than 38.0°C on different days; haemoglobin changes between days 0, 14, and 28; asexual parasite clearance time defined as the time, in days, from randomisation to two consecutive negative blood slides (obtained on different days).

Safety endpoints were defined as adverse events in children who had received at least one dose of the study medication. An adverse event was defined as any untoward medical occurrence, regardless of its relation to the study medication, in accordance with the International Conference of Harmonisation guidelines (ICH). Investigators used clinical judgment to assess causality, and the association was defined as definitely unrelated, unlikely to be related, possibly related, probably related, and definitely related. Only adverse events that were assessed as at least possibly related to the study drug were considered. These events were grouped according to the WHO adverse-reaction terminology (WHO-ART).

Statistical analysis

We hypothesised that all three treatment groups would have similar efficacy and would be superior to 90%. The PCR-adjusted efficacy in any treatment group was assumed to be 95%. Thus, the hypothesis was accepted if the two-sided 95% CI (Wilson's test) fell entirely above 90%. We therefore needed a sample size of 248 children (124 per site) to show with 80% power that the efficacy of re-treatment with the same ACT was at least 90%. Treatment with QnC was intended as the benchmark with half of participants, given that its administration is more demanding, particularly in the context of directly observed treatment (administered three times a day for 7 days). Assuming 15% of children would be unable to be assessed, the required sample size was 714 (357 per site).

Using DataFax (Clinical DataFax Systems Inc, ON, Canada), data recorded on paper case report forms (CRFs) were sent weekly from both sites to a centralised database located at the Infectious Diseases Institute, University of Makerere, Uganda. Most of the data analysis was done with Stata version 11 (Stata Corp, Lakeway, College Station, TX, USA). We used descriptive statistics to summarise the baseline characteristics. We applied haemoglobin concentration of 100 g/L as a cutoff for anaemia as in previous studies.¹⁶ The efficacy analysis was done according to both a per-protocol and a modified intention-to-treat approach. In the modified intention-to-treat approach, children excluded after enrolment were censored at the time of their final examination. We did some post-hoc analyses of Nelson-Aalen cumulative hazard estimates to generate the curves for risk of PCR-adjusted and PCR-unadjusted treatment failure. We compared survival curves by a log-rank test. Children who completed the treatment course were included in a sensitivity analysis with PCR-unadjusted ACPR at day 28

in the modified intention-to-treat population, considering the children missing the treatment outcome as ACPR or treatment failure.

To assess the PCR-adjusted outcomes, recurrent parasitaemia caused by new infections was considered to be ACPR. Safety was analysed in all patients treated, which included all children who were randomly assigned to treatment who received at least one dose of study medication. We compared the percentage of children who had each adverse event between treatment groups. A p value of less than 0.05 was considered significant. Data were pooled as re-treatment (same ACT vs alternative ACT and same ACT vs QnC) or by regimen (AL vs ASAQ and AL vs QnC; see appendix). The asexual parasite clearance time of 154 children was closely monitored every 6 h in both sites, according to the protocol amendment. We analysed the data using the parasite clearance estimator (PCE),¹⁷ which estimated the parasite-clearance half-life. The trial protocol was amended to include estimating parasite clearance half-life every 6 h for evidence of parasitaemia following recommendations from the Worldwide Antimalarial Resistance Network (WWARN).

The study is registered in ClinTrials.gov, NCT01374581, and the Pan African Clinical Trials Registry, PACTR201203000351114. External monitoring for purposes of guaranteeing data quality and good clinical practice (GCP) compliance was done by the Amsterdam Institute for Global Health and Development.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

See Online for appendix

For WWARN see <http://www.wwarn.org/partnerships/study-groups/parasite-clearance-study-group>

	Re-treatment ACT (n=240)	Alternative ACT (n=233)	QnC (n=98)	DR Congo (n=242)	Uganda (n=329)
Mean age, months (SD)	34.4 (12.6)	35.5 (13.3)	36.0 (13.3)	37.4 (13.1)	33.4 (12.7)
Male sex (%)	131 (55%)	115 (49%)	51 (52%)	129 (53%)	168 (51%)
Median weight, kg (IQR)	12.5 (11–14)	12.5 (11–14)	12.5 (11–14)	13.0 (11–14.6)	12.0 (10.5–14)
Mean height, cm (SD)	87.3 (10.1)	88.8 (11.1)	89.0 (11.0)	91.9 (10.4)	85.5 (10.2)
Mean tympanic temperature, °C (SD)	38.0 (1.2)	37.9 (1.1)	38.0 (1.2)	38.1 (1.0)	37.8 (1.2)
Patients with fever, ≥38°C (%)	105 (44%)	99 (43%)	42 (43%)	132 (55%)	114 (35%)
Mean haemoglobin, g/L (SD)	102 (15)	104 (17)	103 (17)	103 (16)	103 (16)
Patients with anaemia (%)	82 (34%)	87 (37%)	35 (36%)	82 (34%)	122 (37%)
Geometric mean for asexual parasites per µl (95% CI)	26776 (21607–33182)	26976 (21509–33840)	27380 (19357–38729)	27520 (22590–33526)	26561 (21782–32387)
Patients with parasitaemia ≥2000 asexual parasites per µL (%)	216 (90%)	212 (91%)	88 (90%)	222 (92%)	294 (89%)
Patients sleeping under mosquito bednets the previous night (%)	123 (51%)	130 (56%)	55 (56%)	135 (56%)	173 (53%)
Patients carrying gametocytes (%)	4 (2%)	4 (2%)	1 (1%)	1 (<1%)	8 (2%)

Table 1: Demographic and baseline clinical characteristics of Quinact study participants at the Lisungi Health Center (DR Congo) and the Kazo Health Center (Uganda), 2012–14

	Re-treatment ACT (%)	Alternative ACT (%)	QnC (%)
Overall			
New infections	56/73 (75%)	65/83 (78%)	14/23 (61%)
Recrudescence	18/73 (25%)	18/83 (22%)	9/23 (39%)
Indeterminate n	15	13	3
ACPR n/N (%; 95% CI)	190/208 (91%, 87.5–95.2)	188/206 (91%, 87.4–95.1)	77/86 (90%, 83.0–96.0)
DRC			
New infections	20/26 (77%)	31/39 (80%)	6/10 (60%)
Recrudescence	6/26 (23%)	8/39 (21%)	4/10 (40%)
Indeterminate n	2	4	2
ACPR n/N (%; 95% CI)	80/86 (93%, 87.6–98.5)	89/97 (92%, 86.2–97.3)	31/35 (89%, 77.8–99.3)
Uganda			
New infections	35/47 (75%)	34/44 (77%)	8/13 (62%)
Recrudescence	12/47 (26%)	10/44 (23%)	5/13 (39%)
Indeterminate n	13	9	1
ACPR n/N (%; 95% CI)	110/122 (90%, 84.4–95.5)	99/109 (91%, 85.4–96.3)	46/51 (90%, 81.9–98.5)

Data are n/N (%) unless otherwise indicated

Table 2: PCR-adjusted treatment outcome of the rescue treatment by day 28 (per-protocol population) for QUINACT study participants at the Lisungi Health Center (DR Congo) and the Kazo Health Center (Uganda), 2012–14

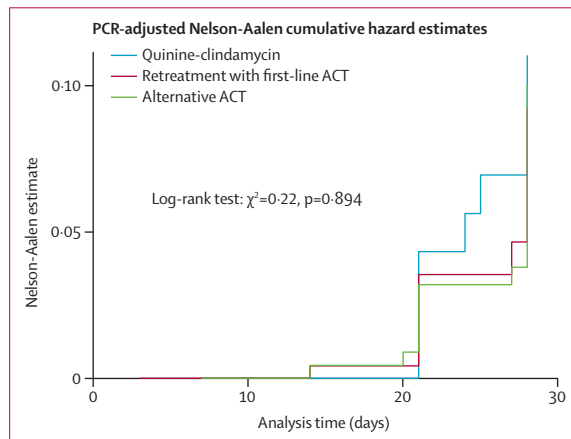


Figure 2: Nelson-Aalen PCR-adjusted estimates for rates of recurrent malaria infection in the modified intention-to-treat population in the re-treatment ACT group, Alternative ACT group, and quinine and clindamycin groups.

Results

From May 22, 2012 (the DR Congo site started on Aug 23, 2012), to Jan 31, 2014, 699 children were assessed for eligibility after having a recurrent infection with the first-line ACT treatment (ASAQ in DR Congo and AL in Uganda; figure 1). Of these children, 571 fulfilled the recruitment criteria. 516 of 571 eligible patients (90%) had parasitaemia of greater than 2000 asexual parasites per μL at recruitment (table 1). 240 children were randomly assigned to receive the re-treatment ACT (95 ASAQ in DR Congo and 145 AL in Uganda), 233 to the alternative ACT (107 AL in DR Congo and 126 ASAQ in Uganda), and 98 to QnC (40 in DR Congo and 58 in Uganda). Treatment

outcomes were available for 223 (93%) children in the re-treatment ACT, for 219 (94%) in the alternative ACT, and for 89 (91%) in the QnC groups (figure 1). The most common reasons for not completing the follow-up were persistent vomiting, inattendance at follow-up, and withdrawal of informed consent. Baseline characteristics were similar across the three treatment groups (table 1). The proportions of children who were excluded from the per-protocol population were similar in the three treatment groups, and their baseline characteristics did not differ from those included in the study. For the assessment of the primary outcome, 15 children assigned to the same ACT, 13 children in the alternative ACT group, and three from the QnC group were excluded because of indeterminate PCR genotyping results (figure 1).

After PCR adjustment, ACPR assessed at day 28 was reached in 190 of 208 patients (91.4%; 95% CI 87.5–95.2) for the re-treatment ACT group, in 188 of 206 patients (91.3%; 87.4–95.1) for the alternative ACT group, and 77 of 86 patients (89.5%; 83.0–96.0) for the QnC group (table 2). The difference in efficacy between the re-treatment ACT and the alternative ACT groups was 0.08% (95% CI –5.5 to 5.7), 1.81% (–4.9 to 10.6) between the re-treatment ACT and the QnC groups, and 1.73% (–5.0 to 10.6) between the alternative ACT and the QnC groups. PCR-unadjusted and PCR-adjusted ACPR values were similar in both study sites after stratification (table 2, appendix). The rates of recrudescence in the three treatment groups were similar (log-rank test: $\chi^2=0.22$, $p=0.894$; figure 2). Within the per-protocol population, 88 children (40 re-treatment ACT, 35 alternative ACT, and 13 QnC treatment groups) still had similar *P falciparum* strains as at the initial enrolment in the pre-trial phase. After rescue treatment, the proportion of persistent recrudescence was distributed in the three groups as follows: five (13%) of 40 children in the re-treatment ACT group, four (11%) of 35 children in the alternate ACT group, and four (31%) of 13 in the QnC treatment group.

The unadjusted ACPR in the per-protocol population was 135 of 223 patients (60.5%; 95% CI 54.1–67.0) for the re-treatment ACT, 123 of 219 patients (56.2%; 49.6–62.8) for the alternative ACT, and 63 of 89 patients (70.8%; 61.3–80.3) for the QnC group (appendix). No treatment failure occurred before day 14. The sensitivity analysis in the modified intention-to-treat population showed that the proportion of patients with PCR-unadjusted ACPR was 135 of 236 patients (57.2%; 95% CI 50.8–63.5) when the treatment given to children with an unknown efficacy endpoint was considered as a treatment failure and was 145 of 236 (61.4%; 55.2–67.7) when treatment was considered as successful in the re-treatment ACT group. In the group given an alternative ACT, the ACPR ranged from 123 of 230 patients (53.5; 47.0–59.9) to 132 of 230 (57.4%; 50.9–63.8), with ACPR values ranging from 63 of 93 patients (67.7%; 58.2–77.3) to 66 of 93 (71.0%; 61.7–80.3) in the group treated with QnC. Malaria recurrent infection tended to occur later in

the patients assigned to QnC, compared with the other groups (log-rank test $\chi^2=5.73$, $p=0.056$; appendix).

Fever resolution was less rapid in children assigned to QnC treatment than in children assigned to either ACT treatment group (appendix). The proportion of children with parasitaemia on days 1, 2, and 3 was higher in the QnC group than in either of the ACT treatment groups. On day 7, all children had negative blood smears. The parasite clearance trend can be seen in the appendix. Children given QnC had a longer half-life parasite clearance (mean 6.2 h; SD 2.1), than did those treated with AL (2.7 h; 1.0) or ASAQ (2.4 h; 1.3; both $p<0.0001$), although this parasite clearance was similar between the ACT groups ($p=0.40$). The observation was similar when stratified by site, although the QnC group was under-represented.

On day 28, the mean haemoglobin increased by 12 g/L (SD 17) in the re-treatment ACT, 11 g/L (18) in the alternative ACT, and 16 g/L (14) in the QnC groups (appendix). When pooled by regimen, the mean increase was 11 g/L (18) for AL and 12 g/L (18) for ASAQ (appendix). None of the differences between mean increases were significant.

Pooling data by regimen, 252 children were assigned to AL and 221 were assigned to ASAQ. The baselines for the two groups were similar (appendix). The PCR-unadjusted ACPR value for AL was 133 of 236 (56.4; 95% CI 50.0–62.7), and 125 of 206 (60.7%; 53.9–67.4) for ASAQ (appendix). The PCR-adjusted ACPR was similar for both AL and ASAQ treatment groups (199 of 219; 90.9%; 87.0–94.7 vs 179 of 195; 91.8%; 87.9–95.7) with a log-rank test (χ^2) value of 0.20 ($p=0.903$; appendix). The percentage of children with fever on days 1, 2, and 3 was lower in the ASAQ group than the AL and QnC treatment groups (appendix). The PCR-unadjusted ACPR was similar to the unadjusted values (appendix). The site-specific cumulative hazard estimates are presented in the appendix.

Overall, 235 adverse events were observed (table 3). The most common events (>5%) reported in the re-treatment ACT group were anorexia, asthenia, coughing, abnormal behaviour, and diarrhoea. Anorexia was the most frequently reported adverse event in the alternative ACT group, although anorexia, abnormal behaviour, asthenia, and pruritus were the most commonly reported after treatment with QnC. None of the adverse events were considered serious. The group assigned to alternative ACT had fewer drug-related adverse events than the re-treatment ACT group ($p<0.0001$) and the QnC treatment group ($p<0.0001$).

66 adverse events were observed in the AL group, and 121 in the ASAQ group. The most commonly reported adverse events were abdominal pain, abnormal behaviour, anorexia, asthenia, and coughing in the ASAQ group, and anorexia and diarrhoea were the most commonly reported in the AL group (appendix). One case of either hypoglycaemia or convulsions occurred in the QnC group, and one case of abnormal urine (verbatim

	Re-treatment ACT n=240 (%)	Alternative ACT n=233 (%)	QnC n=98 (%)
Abdominal pain	8 (3%)	10 (4%)	1 (1%)
Abnormal behaviour	13 (5%)	3 (1%)	6 (6%)
Allergic reaction	1 (<1%)	0 (0%)	0 (0%)
Anaemia	0	0 (0%)	1 (1%)
Anorexia	31 (13%)	13 (6%)	12 (12%)
Asthenia	20 (8%)	2 (1%)	6 (6%)
Bradycardia	1 (<1%)	0 (0%)	0 (0%)
Bronchitis	1 (<1%)	0 (0%)	0 (0%)
Convulsions	0 (0%)	0 (0%)	1 (1%)
Coughing	16 (7%)	6 (3%)	0 (0%)
Diarrhoea	12 (5%)	9 (4%)	2 (2%)
Dizziness	0 (0%)	1 (<1%)	1 (1%)
Headache	0 (0%)	0 (0%)	1 (1%)
Hypoglycaemia	0 (0%)	0 (0%)	1 (1%)
Jaundice	1 (<1%)	1 (<1%)	0 (0%)
Musculoskeletal pain	1 (<1%)	0 (0%)	0 (0%)
Nausea	5 (2%)	3 (1%)	4 (4%)
Periorbital oedema	0 (0%)	1 (<1%)	0 (0%)
Pruritus	9 (4%)	1 (<1%)	5 (5%)
Rales	1 (<1%)	0 (0%)	0 (0%)
Rash	2 (1%)	1 (<1%)	1 (1%)
Splenomegaly	0 (0%)	1 (<1%)	0 (0%)
Tachycardia	0 (0%)	1 (<1%)	2 (2%)
Tinnitus	0 (0%)	0 (0%)	2 (2%)
Urine abnormal	1 (<1%)	1 (<1%)	0 (0%)
Vomiting	8 (3%)	2 (1%)	2 (2%)
Total	131	56	48

Table 3: Summary of adverse events occurring up to day 28 in patients having received at least one dose (n=571) for QUINACT study participants at the Lisungi Health Center (DRC) and the Kazo Health Center (Uganda), 2012–14

“dark urine”) was reported in the ASAQ group and in the AL group. Of all adverse events reported, ten (6%) were graded as severe. AL was better tolerated, compared with QnC ($p=0.0005$) and ASAQ ($p<0.0001$).

Discussion

The PCR-adjusted efficacy of the three different rescue treatments tested was similar. Treating a patient with a recurrent *P falciparum* infection with the same treatment used for the primary episode had similar efficacy to other options, and this should be considered as a possible approach for rescue treatment. However, its effect on the selection of *P falciparum* resistant strains should be monitored.

The PCR-unadjusted efficacy of QnC tended to be higher than the efficacy of the other two groups. This observation is probably related to the length of treatment—7 days for QnC compared with 3 days for the other two treatments. However, in both geographical sites, the proportion of recrudescence seemed to be higher in the QnC group, though the sample size is too

small to estimate the actual efficacy of QnC by site. In previous trials in Uganda, the proportion of PCR-adjusted treatment failure after treatment with quinine was 11.4% in Tororo¹¹ and 69% in Kampala.⁵ It is worth noting that the study by Achan and colleagues⁵ showed only an estimation of quinine effectiveness as the treatment was not directly observed and the study reflects poor treatment adherence. There is a paucity of data on the efficacy of quinine as a treatment for uncomplicated malaria in DR Congo.

Despite good PCR-adjusted efficacy and tendency for a long post-treatment prophylactic period, QnC might not be suitable for rescue treatment for several reasons, including the long duration of treatment and poor tolerance, which can affect adherence, thereby resulting in poor effectiveness.^{5,18} Additionally, the availability of clindamycin is restricted in most sub-Saharan settings, particularly for children.^{19,20} Clindamycin can also induce antibiotic-associated diarrhoea and its use on a large scale in malaria treatment (in combination with quinine) could contribute to the spread of bacterial resistance. The paediatric formulation (granules for oral suspension in sucrose, ethyl parahydroxybenzoate, poloxamer, and artificial cherry oil) is unstable and requires storage at 25°C or less, which poses a challenge in many endemic areas.

In a study done in Uganda, Yeka and colleagues¹¹ reported that alternative ACT is a better rescue treatment than quinine. However, the option of re-treating with the same ACT used for the primary episode was not considered. The risk of recurrent infections was much higher in Tororo, where the study was done, than that observed in Kazo, a difference related to the much higher intensity of transmission.

The proportion of children with detectable parasitaemia on days 1, 2, and 3 was higher in the QnC group than in the ACT groups. The added value of clindamycin to the reduction in asexual parasite clearance time with quinine is uncertain.^{19,20} Two children (1%) of 135 in the AL group in Uganda had microscopically detectable parasitaemia on day 3 after re-treatment. This outcome is not common with ACT.² In DR Congo, all children randomly assigned to the ACT groups cleared parasites by day 3. Close monitoring (every 6 h) showed a similar parasite clearance half-life for ASAQ and AL, but much longer for QnC. The number of children involved in this analysis was small as this study procedure was added as a protocol amendment during the study. It is important to provide more data on the parasite clearance estimations, since these data would provide relevant information for the surveillance of artemisinin resistance.²¹ It is worth mentioning that with almost 1000 samples obtained in the DR Congo site, we contributed to the publication by the Karma consortium, in which it is indicated that K13 resistance is most probably not present in DR Congo.²²

The group re-treated with the same ACT had more adverse events because of the higher proportion of adverse events in the DR Congo site. In principle, re-treatment with the same ACT does not reduce the safety profile.^{23,4} The adverse events observed in the group treated with QnC are likely to be caused by quinine, given that clindamycin is reported to be well tolerated.¹⁹ The group assigned to AL treatment had fewer adverse events than the groups given ASAQ or QnC. In general, both AL and ASAQ are well tolerated.^{4,24-26}

This study has some limitations. The sample size calculation was based on the assumption of a 95% efficacy for the three rescue treatments, which required the lower limit of the 95% CI to be above 90%. However, the point estimate of the PCR-adjusted efficacy of all treatments was lower than expected. Therefore, both ACTs had a similar 95% CI with a lower limit of 87.5% for the same ACT and 87.4% for the alternative ACT groups. The availability of resistance marker results could provide additional arguments for or against re-treatment with the same ACT although this information could be acquired in a classic randomised trial design. Because of potential residual submicroscopic parasitaemia that could persist after ACT treatment, the conventional PCR-adjustment used in this study might have some limitations in the classification of new infections and recrudescence, which might be avoided by the use of quantitative PCR.²⁷

Few trials have investigated the rescue treatment of uncomplicated malaria. This study provides evidence on the 28-day efficacy of re-treatment with the same ACT, a relatively common practice. The study also contributes to the scarce information on the efficacy of QnC. The high proportion of new infections is an indicator of the high malaria transmission in the study areas. The failure to clear recrudescence parasites seemed to be similar in both ACT groups, but higher in the QnC group. The number of patients in this subset might not allow making a conclusive statement in terms of significance. A study including a population aged more than 5 years and a longer follow-up period would provide more evidence for the generalisability of these results. The 42 days of passive follow-up in pre-trial phase and a shorter 28 days of active follow-up in the study phase was necessary to protect the quality of data and avoid the overcrowding of study sites.

Conclusion

If we consider the poor effectiveness and tolerability of quinine, the reduced availability of the paediatric formulation of clindamycin, and the availability of only one first-line ACT at the community level in many settings, we conclude that a rescue treatment with the same ACT in correct doses as recommended could be an alternative option to quinine or the not always available alternative ACT. More evidence is needed before assessing the generalisability of this statement and caution is paramount when re-treating with the first-line ACT in areas where resistance to the partner drug has been confirmed.

Contributors

HT, JPVg, PL, UDA, and HMM initiated the idea. HMM, HT, PL, and JPVg wrote the study protocol. HMM, CN, RIdL, AK, JPVg, and PL were involved in the acquisition of data. VB and AR-U supervised and interpreted molecular analysis. HMM and JPVg analysed and interpreted the data. HMM drafted the manuscript. All authors contributed to the writing of the paper, and approved the final version.

Declaration of interest

We declare no competing interests.

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References

- WHO. World malaria report 2015. Geneva: World Health Organization, 2015. <http://www.who.int/malaria/media/world-malaria-report-2015/en/> (accessed May 27, 2016).
- WHO. Guidelines for the treatment of malaria. Geneva: World Health Organization, 2006.
- Wembonyama S, Mpaka S, Tshililo L. Medicine and health in the Democratic Republic of Congo: from independence to the third republic. *Med Trop* 2007; **67**: 447–57 (in French).
- Yeka A, Lameyre V, Afizi K, et al. Efficacy and safety of fixed-dose artesunate-amodiaquine vs artemether-lumefantrine for repeated treatment of uncomplicated malaria in Ugandan children. *PLoS One* 2014; **9**: e113311.
- Achan J, Tibenderana JK, Kyabayinze D, et al. Effectiveness of quinine versus artemether-lumefantrine for treating uncomplicated *falciparum* malaria in Ugandan children: randomised trial. *BMJ* 2009; **339**: b2763.
- Kofoed PE, Mapaba E, Lopes F, Pussick F, Aaby P, Rombo L. Comparison of 3, 5 and 7 days' treatment with Quinimax for *falciparum* malaria in Guinea-Bissau. *Trans R Soc Trop Med Hyg* 1997; **91**: 462–64.
- Yeka A, Achan J, D'Alessandro U, Talisuna AO. Quinine monotherapy for treating uncomplicated malaria in the era of artemisinin-based combination therapy: an appropriate public health policy? *Lancet Infect Dis* 2009; **9**: 448–52.
- Fungladda W, Honrado ER, Thimasarn K, et al. Compliance with artesunate and quinine + tetracycline treatment of uncomplicated *falciparum* malaria in Thailand. *Bull World Health Organ* 1998; **76** (suppl 1): 59–66.
- Zwang J, Olliaro P, Barennes H, et al. Efficacy of artesunate-amodiaquine for treating uncomplicated *falciparum* malaria in sub-Saharan Africa: a multi-centre analysis. *Malar J* 2009; **8**: 203.
- Tshefu AK, Gaye O, Kayentao K, et al. Efficacy and safety of a fixed-dose oral combination of pyronaridine-artesunate compared with artemether-lumefantrine in children and adults with uncomplicated *Plasmodium falciparum* malaria: a randomised non-inferiority trial. *Lancet* 2010; **375**: 1457–67.
- Yeka A, Tibenderana J, Achan J, D'Alessandro U, Talisuna AO. Efficacy of quinine, artemether-lumefantrine and dihydroartemisinin-piperazine as rescue treatment for uncomplicated malaria in Ugandan children. *PLoS One* 2013; **8**: e53772.
- Muhindo Mavoko H, Kalabuanga M, Delgado-Ratto C, et al. Uncomplicated clinical malaria features, the efficacy of artesunate-amodiaquine and their relation with multiplicity of infection in the Democratic Republic of Congo. *PLoS One* 2016; **11**: e0157074.
- Snounou G, Beck HP. The use of PCR genotyping in the assessment of recrudescence or reinfection after antimalarial drug treatment. *Parasitol Today* 1998; **14**: 462–67.
- WHO. Methods and techniques for clinical trials on antimalarial drug efficacy: genotyping to identify parasite populations: informal consultation organized by the Medicines for Malaria Venture. Amsterdam: World Health Organization, 2007.
- WHO. Assessment and monitoring of antimalarial drug efficacy for the treatment of uncomplicated *falciparum* malaria. Geneva: World Health Organization, 2003.
- WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Geneva: World Health Organization, 2004.
- Flegg JA, Guerin PJ, White NJ, Stepniewska K. Standardizing the measurement of parasite clearance in *falciparum* malaria: the parasite clearance estimator. *Malar J* 2011; **10**: 339.
- WHO. Guidelines for the treatment of malaria 3rd edn. Geneva: World Health Organization, 2015.
- Lell B, Kremsner PG. Clindamycin as an antimalarial drug: review of clinical trials. *Antimicrob Agents Chemother* 2002; **46**: 2315–20.
- Obonyo CO, Juma EA. Clindamycin plus quinine for treating uncomplicated *falciparum* malaria: a systematic review and meta-analysis. *Malar J* 2012; **11**: 2.
- WWARN Parasite Clearance Study Group. Baseline data of parasite clearance in patients with *falciparum* malaria treated with an artemisinin derivative: an individual patient data meta-analysis. *Malar J* 2015; **14**: 359.
- Ménard D, Khim N, Beghain J, et al. A worldwide map of *Plasmodium falciparum* K13-propeller polymorphisms. *N Engl J Med* 2016; **374**: 2453–64.
- Sagara I, Fofana B, Gaudart J, et al. Repeated artemisinin-based combination therapies in a malaria hyperendemic area of Mali: efficacy, safety, and public health impact. *Am J Trop Med Hyg* 2012; **87**: 50–56.
- Shayo A, Buza J, Ishengoma DS. Monitoring of efficacy and safety of artemisinin-based anti-malarials for treatment of uncomplicated malaria: a review of evidence of implementation of anti-malarial therapeutic efficacy trials in Tanzania. *Malar J* 2015; **14**: 135.
- Yavo W, Konaté A, Kassi FK, et al. Efficacy and safety of artesunate-amodiaquine versus artemether-lumefantrine in the treatment of uncomplicated *Plasmodium falciparum* malaria in sentinel sites across Cote d'Ivoire. *Malar Res Treat* 2015; **2015**: 878132.
- Onyamboko MA, Fanello CI, Wongsan K, et al. Randomized comparison of the efficacies and tolerabilities of three artemisinin-based combination treatments for children with acute *Plasmodium falciparum* malaria in the Democratic Republic of the Congo. *Antimicrob Agents Chemother* 2014; **58**: 5528–36.
- Beshir KB, Sutherland CJ, Sawa P, et al. Residual *Plasmodium falciparum* parasitemia in Kenyan children after artemisinin-combination therapy is associated with increased transmission to mosquitoes and parasite recurrence. *J Infect Dis* 2013; **208**: 2017–24.