HIGH REINFECTION RATE AND TREATMENT FAILURES IN CHILDREN TREATED WITH AMODIAQUINE FOR FALCIPARUM MALARIA IN MUHEZA VILLAGES, NORTHEASTERN TANZANIA

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Abstract. In May 2003, we studied amodiaquine (AQ) efficacy in children < 5 years of age with uncomplicated falciparum malaria in Magoda and Mpapayu (with insecticide treated nets [ITNs]) and Mgome (without ITNs) in Muheza, Tanzania. The trial involved 28 days of follow-up, and data were adjusted by polymerase chain reaction (PCR) genotyping of msp1 and msp2 genes. Additionally, Pfcr1 codon 72–76 polymorphisms were studied by PCR and sequence-specific oligonucleotide probe (SSOP) ELISA. In 54 cases with complete follow-up, a significant difference in late treatment failure (LTF) rates was seen (60.7% in ITN versus 88.5% in non-ITN villages, \( P = 0.02 \)) before PCR correction. However, after PCR correction, 23 cases (60.5%) were confirmed as reinfections, giving a true LTF rate of 21.4% (6/28) and 34.6% (9/26) in the above settings, respectively. Frequency of Pfcr1 CVIET haplotype mutation pretreatment was high (97.0%); the remaining samples were CVKNK. We conclude that AQ alone is no longer effective in the study area.

INTRODUCTION

The emergence and spread of chloroquine (CQ)- and sul- fadoxine/pyrimethamine (S/P)-resistant Plasmodium falciparum poses a serious problem for the treatment of malaria in many regions of the world.1 Amodiaquine (AQ) and CQ are structurally related 4-aminoquinolines. However, AQ has been evaluated in a number of clinical trials and found to be safe and highly effective in clearing P. falciparum infections, even in areas with high CQ resistance.2–4 Thus, AQ is now considered as an alternative option to CQ and S/P for the management of uncomplicated P. falciparum malaria in Africa.5 In areas where falciparum malaria is highly endemic, the reappearance of asexual erythrocytic stages of P. falciparum after a correct therapeutic dose may be attributed to recrudescence.6 The in vivo response is influenced by the parasite’s resistance profile and host immunity; the latter is age dependent.7–9

In our effort to define the epidemiology of drug-resistant parasites, it is important to distinguish between recrudescence and new infections. Therefore, molecular techniques to differentiate between the two outcomes are often included in efficacy trials as recommended by the World Health Organization (WHO).10 This may be particularly important in areas of high malaria transmission.11

Studies of the correlation between drug resistance in natural populations and genetic polymorphisms linked to drug resistance in P. falciparum may allow the development of molecular tools to help predict responses to antimalarials.12 The P. falciparum chloroquine resistance transporter gene (Pfcr1) is a 13-exon gene in which up to eight codons differ between the CQ-sensitive and the CQ-resistant reference clones.13 Of these codons, a point mutation from lysine to threonine at position 76 (K76T) plays a key role in CQ resistance in vitro and in vivo.13,14 In Africa, wild type isolates exist as the CVKNK haplotype in codon 72–76, whereas the CQ-resistant mutant is the CVIET haplotype. The molecular mechanisms of AQ resistance have not been fully addressed, but the similarity between CQ and AQ in their chemical structure, their likely common modes of action,15,16 and some apparent cross-resistance suggests that molecular markers selected as a function of CQ use might also compromise effectiveness of AQ.15,17 Recently, it has been reported that there is an association between the K76T mutation and AQ resistance in vivo.18 Another study in Tanzania,19 where high CQ resistance is found, reported a high frequency of the K76T mutation (76) pre-CQ treatment. In 2002, 25 children 6–59 months of age living in Magoda and Mpapayu villages (with insecticide treated nets [ITNs]) in Muheza district, Tanzania, were given AQ (three divided doses over 3 days). All 25 children had cleared their infection by day 7, and all except 1 were parasite free on day 14 of follow-up. Indeed, all cases were classified as having adequate clinical response (ACR) by day 14 of follow-up.

As a follow-up to the 2002 study, we evaluated the efficacy of AQ alone in 2003 in the two villages and a neighboring village of Mgome (without ITNs) to see if earlier efficacy has been maintained. This time, the follow-up period was extended to 28 days as recommended by the WHO.10 The aim of this study was to assess the efficacy of AQ in children < 5 years of age in an area where high levels of CQ and SP resistance prevail and include adjustment of recrudescence and reinfection after AQ treatment by polymerase chain reaction (PCR). Furthermore, we also assessed the relationship between the Pfcr1 codon 72–76 haplotypes and AQ treatment outcome.

MATERIALS AND METHODS

Study area and population. Magoda and Mpapayu villages are separated by 1 km. They are located at 5°11′ S and 38°52′ E in the Muheza district, where malaria transmission is intense and perennial. The inhabitants depend on subsistence farming, growing oranges, maize, and cassava for cash crops. Details of the Magoda village have been given previously.21 All permanent residents in Magoda and Mpapayu had ITNs distributed to them free of charge in 1998 and 2001, respectively.20 In addition, there is a mobile clinic that oper-
Similarly, a high occurrence of mutations in the P. falciparum dihydrofolate reductase (dhfr) and dihydropterotae synthetase (dhps) genes that confer SP resistance has previously been observed in the Magoda and Mpapayu villages.\textsuperscript{24,25} Mgabe village is 2 km from Mpapayu and has no ITNs in place.

Screening for malarial parasites, anemia, and selection of cases. All children 6–59 months of age were screened for malarial parasites on day −1 at the mobile clinic in the villages. Axillary temperature was taken, and completion of a morbidity questionnaire for each child was done. Thick and thin blood smears were taken for malaria diagnosis. A sample of capillary blood (300–500 \(\mu\)L) was collected into EDTA tubes from all children for other laboratory tests. Blood samples were also drawn into heparinized capillary tubes for estimation of anemia by packed cell volume (PCV). EDTA blood was centrifuged to obtain plasma and packed red cells and were both stored at −20°C until use. For PCR, the packed red blood cells were thawed, and 50 \(\mu\)L was spiked onto a filter paper strip (Whatman no. 3). Children with pure \textit{P. falciparum} infection, a history of fever for the past 1–2 days, or measured fever (\(\geq 37.5°C\)), and at least 2,000 asexual parasitaemia/\(\mu\)L were selected for admission into the study if they met the inclusion criteria, which included consent from parents/guardians. Children with danger signs, other febrile illnesses, severe anemia (PCV < 15\%), and those not consenting to participate were excluded.

Drug administration. All enrolled children were treated orally under medical supervision with amodiaquine (25 mg/kg body weight) given as three divided doses over 3 days (10 mg/kg body weight on days 0 and 1, 5 mg/kg on day 2). Subjects were observed after each dose, and a full dose was repeated if vomiting occurred within 30 minutes. Paracetamol was also administered to all cohort children on day 0, and parents were advised to give tepid sponging at home in case of fever. Amodiaquine tablets used were Emoquine (Elys Chemical Industries Ltd., Nairobi, Kenya). The tablets passed quality assurance tests for quantity (104.5 ± 2.6\%) compared with United States Pharmacopeia (USP) (93.0–107.0\%) and dissolution (86.9\% in 30 minutes) compared with USP (75\% in 30 minutes). The test was performed by D. Hipolite Shewiyo of the Tanzania Food and Drug Administration (TFDA) and Dr. Omari Minzi of Muhimbili University College of Health Sciences (MUCHS).

Follow-up and treatment outcome. Follow-up criterion was based on the WHO protocol of an extended 28-day \textit{in vivo} test.\textsuperscript{30} In brief, the children were monitored clinically, and blood samples for parasite determination were obtained daily from day 0 until day 3 and then again on days 7, 14, 21, and 28. Response to treatment was classified as early treatment failure (ETF), late treatment failure (LTF), and adequate clinical and parasitological response (ACPR) according to WHO.\textsuperscript{30} Mothers were advised to come back with their children during unscheduled days in case of fever or deteriorating clinical conditions. PCV was estimated for all children before admission into the study and on days 14, 21, and 28 of follow-up.

DNA extraction. DNA extraction from blood spots on filter paper was carried out by the chelex-100 method as previously described\textsuperscript{26} with some modifications.\textsuperscript{27} In brief, all samples were extracted in a 96-well plate format. The blood spot was first soaked in phosphate-buffered saline (PBS) with 0.5\% saponin overnight and was washed twice in 1 mL of PBS. The segment was boiled for 8 minutes in 100 \(\mu\)L of H\(_2\)O and 50 \(\mu\)L of 20\% chelex, suspended in distilled water (pH 9.5), and centrifuged at 5,000 rpm for 10 minutes.

\textit{Msp1} and \textit{msp2} PCR. To differentiate whether the recrudescence parasite population is resistant and persisting from the initial infection or the parasites were from a new infection, a nested PCR amplification of the polymorphic regions of \textit{P. falciparum} genes \textit{msp1} (K1, MAD20 and RO33) and \textit{msp2} (IC1 and FC27) was performed as described elsewhere.\textsuperscript{28} One microliter of the extracted DNA suspension was used in the outer PCR reactions. The nested PCR products (10 \(\mu\)L) were run in electrophoresis on 2–2.5\% metaphor agarose gels in 1× Tris 89 mM, EDTA 2 mM, Boric acid 89 mM (TBE) buffer, stained with ethidium bromide, and visualized in UV transillumination. A 50-bp DNA molecular weight marker was used to size the bands. Interpretation of \textit{msp1} and \textit{msp2} data was based on definitions given elsewhere.\textsuperscript{29}

\textit{Pfcrt} PCR. A nested PCR\textsuperscript{31} described in detail at http://medschool.umaryland.edu/CVD/nejm2001djimde.htm was used to amplify fragments of the \textit{Pfcrt} gene. The only modifications in the procedures were that the TCRD2 primer for \textit{Pfcrt} nested PCR was biotinylated at the 5′-end by the supplier (MWG Biotech, Risikov, Denmark). The 20-\(\mu\)L \textit{Pfcrt} outer PCR reaction mix consisted of 0.2 mmol/L of each dNTPs, 1 \(\mu\)mol/L of the primer set TCRP1/TCRP2, 1.25 U of DNA Qiagen HotStart polymerase (Qiagen, Albertslund, Denmark), buffer containing 2.5 mmol/L MgCl\(_2\), and 1 \(\mu\)L extracted DNA. The reaction mix of the nested \textit{Pfcrt} PCR was identical to the outer PCR, and the primer set TCRD1/TCRD2 was used. The conditions of the outer and nested \textit{Pfcrt} PCR were as described elsewhere.\textsuperscript{30} Amplifications were performed in 96-well PCR plates, and the reaction mix was overlaid with one drop of mineral oil.

\textit{Pfcrt} sequence-specific oligonucleotide probe ELISA. The sequence-specific oligonucleotide probe (SSOP) ELISA was performed as described recently.\textsuperscript{31} In brief, the nested \textit{Pfcrt} PCR products were diluted 1:10 in H\(_2\)O in a 96-well PCR plate, denatured at 95°C for 5 minutes, and immediately thereafter cooled to 4°C until use. One hundred microliters of cold dilution buffer (1× PBS with 0.05% Tween-20) and 2 \(\mu\)L of the diluted PCR products were added to each well of the streptavidin-coated ELISA plates. Replicate ELISA plates were made, enabling simultaneous probing with SSOPs targeting the full panel of \textit{Pfcrt} haplotypes. One hundred microliters of the 3-end digoxigenin conjugated SSOPs of the CVMNK, CVIET, and SVMNT haplotypes (MWG Biotech, Risikov, Denmark) in tetra-methyl-ammonium chloride (TMAC; Sigma Aldrich Chemie, Seelze, Germany) was added to each well and incubated in a hybridization oven (AH Diagnostics, Aarhus, Denmark) at 53°C. Hereafter followed two rounds of washing and incubation in TMAC solution two times for 10 minutes each at 60°C. Peroxidase conjugated anti-digoxigenin antibody in dilution buffer (1:1,000; Roche Diagnostics, Mannheim, Germany) was added to each
well. After incubation, o-phenylene-diamine (OPD) solution of 1.5 mg/mL of 1,2-phenyldiamine dihydrochloride (DAKO, Glostrup, Denmark) dissolved in water containing 0.015% H₂O₂ was added to the plates for 30 minutes, followed by adding 1.25 mol/L H₂SO₄ to stop the reaction. The optical density at 492 nm was measured in an ELISA reader. The scoring of data was performed as described elsewhere.\(^3\)

**Ethical considerations.** Ethical clearance for this study was obtained from the Medical Research Coordinating Committee of the National Institute for Medical Research. One week before the study, the village leadership was informed of our study. The study aims and procedures were explained to the parents/guardians, and informed consent was obtained before screening of children for malaria. Further consent was sought from parents/guardians of those that qualified to enter into the efficacy trial. It was clearly stated that participation is free, and the children could withdraw from the study at any time, and that if one declines, s/he will still have access to the free medical care provided to all community members.

**Statistical analysis.** Data management and analysis was done using EPI-Info version 6.04b and STATA version 7.0 Statistical Software. Comparison of means used Student’s t test. Proportions were compared by the \(\chi^2\) test. \(P < 0.05\) were considered significant.

**RESULTS**

**Malarial parasites, anemia, and treatment outcome.** A total of 269 children 6–59 months of age were screened for malarial parasites at a mobile clinic in the villages. Overall, parasites were seen in 131 (48.7%) of the examined children. Significantly higher prevalence of malaria was seen in the village of Mgome without ITNs (non-ITN village); 65/78 (83.3%) compared with the ITN villages (Magoda and Mpapayu); 66/191 (34.6%; \(\chi^2 = 50.74, P < 0.001\)). Screening data from the two ITN villages revealed significantly higher parasite prevalence at Magoda (41.6%) compared with Mpapayu (25.0%; \(\chi^2 = 5.69, P = 0.017\)). However, no significant difference was seen in age, body temperature, or parasite density between these villages (data not shown). The two ITN villages were thus considered jointly and were compared with the single non-ITN village Sixty of the 131 malaria-positive cases met the inclusion criteria: 28 from ITN villages and 26 from the non-ITN village (Table 1). Baseline features of the cohort children were similar between the two village settings.

A higher parasite clearance was seen in the ITN villages compared with the non-ITN villages between days 21 and 28 (Figure 1). Similar rates were seen in the first 14 days; on day 3 of follow-up, parasite-free cases were 64.3% in the ITN and 69.2% in the non-ITN villages, and by day 7, all cases in the ITN villages were parasite free and only one of the cases in the non-ITN village was parasitemic. Testing of all day 7 slide-negative samples by nested \(msp2\) PCR confirmed they were indeed all parasite negative. Parasite-free cases on day 14 were as follows: ITN villages, 19/28 (67.9%); non-ITN village, 20/26 (76.9%). However, a significantly higher proportion were parasite free on day 21 in the ITN villages compared with the non-ITN village (\(\chi^2 = 9.74, P = 0.002\)). A similar trend was seen on day 28, but this was not statistically significant (\(\chi^2 = 3.08, P = 0.079\)).

Of 54 children with successful 28-day follow-up, 40 (74.1%) had parasitemia between days 14 and 28, whereas 14 were aparasitemic within the 28-day period. After PCR correction, 23 of 38 (60.5%) parasiticemias (two could not be classified) were found to be reinfections. Before PCR adjustment, adequate clinical and parasitological responses (ACPRs) were seen in 39.3% and 11.5% of the cases from ITN and non-ITN villages, respectively (Table 2), and a significant higher LTF (\(\chi^2 = 5.405, P = 0.02\)) was observed in the non-ITN village (88.5%) compared with the ITN villages (60.7%). After PCR correction, only 15 (6 in ITN and 9 in non-ITN villages) of 40 LTF cases were true recurrent infections. Among the 40 LTF cases, there were 18 late clinical failures (LCFs); 9 cases from each setting seen between days 14 and 28. The LCF cases had fever and parasitemia on the treatment failure day mainly because of new infections and were given alternative treatment at the village, but two cases were referred to the Muheza district hospital for quinine and further case management. At the end of the 28-day follow-up, the remaining 22 cases were late parasitological failures (LPFs); 8 cases (1 new infection) from ITN villages and 14 cases (9 new infections) in the non-ITN village. Comparison of hematocrit data revealed an increase in the mean PCV in both ITN and non-ITN villages. Both village settings had similar PCV levels.

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**TABLE 1**

Baseline characteristics for 54 children treated with AQ for uncomplicated *falciparum* malaria in a 28-day *in vivo* test in ITN and non-ITN villages in Muheza, northeastern Tanzania

<table>
<thead>
<tr>
<th></th>
<th>ITN (N = 28)</th>
<th>Non-ITN (N = 26)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (months, SD)</td>
<td>31.2 (13.7)</td>
<td>30.3 (15.7)</td>
<td>0.8293</td>
</tr>
<tr>
<td>Mean weight (kg, SD)</td>
<td>11.4 (2.7)</td>
<td>11.6 (3.1)</td>
<td>0.7813</td>
</tr>
<tr>
<td>Mean temperature (°C, SD)</td>
<td>37.0 (1.1)</td>
<td>37.4 (1.1)</td>
<td>0.2122</td>
</tr>
<tr>
<td>Temperature range (°C)</td>
<td>36.0–40.6</td>
<td>36.0–39.9</td>
<td>–</td>
</tr>
<tr>
<td>Median parasite density (rings/µL, with 25th, 75th percentile)</td>
<td>7,943 (3,981, 15,549)</td>
<td>12,589 (3981, 19,953)</td>
<td>–</td>
</tr>
</tbody>
</table>

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**Figure 1.** Proportion parasite free (%) in children treated with AQ for uncomplicated *falciparum* malaria in a 28-day *in vivo* test in ITN and non-ITN villages in Muheza, northeastern Tanzania. *Significantly* higher proportion of parasite-free children in ITN compared with non-ITN villages on day 21 of follow-up (\(\chi^2 = 9.74, P = 0.002\)). There was a higher proportion of parasite-free children in ITN compared with non-ITN villages on day 28, but this was not significant (\(\chi^2 = 3.08, P = 0.079\)).
when within-day comparisons were made on all four occasions (Table 3), and there was a significant gain in PCV on day 14 compared with baseline in both settings (ITN: \( t = 4.0959, P = 0.0004 \); non-ITN: \( t = 3.9403, P = 0.0006 \)). Furthermore, there was a significant gain in PCV on day 21 compared with baseline in ITN villages only (ITN: \( t = 2.9705, P = 0.0065 \); non-ITN: \( t = 0.3312, P = 0.7432 \)).

**PCR analysis of the Pfcrt haplotypes.** Codon 72–76 haplotype data from *P. falciparum* CQ resistance transporter (*Pfcrt*) gene analysis at day 0 and after treatment revealed 34 (82.9%) paired samples were single CVIET haplotype pre- and post-treatment (irrespective of PCR adjustment), whereas the remaining 7 samples were different. Two of the 7 samples were mixed CVIET/CVMNK infections with CVIET in majority before treatment, but single CVIET haplotype infections at day 21 after treatment. The other four were mixed before treatment but single CVIET haplotype after treatment. Only one sample was CVMNK haplotype pre-treatment and mixed (without a clear majority infection) after treatment at day 14 and CVIET at day 21. However, two of the samples were found to be new infections when adjusted by PCR.

**DISCUSSION**

AQ has been evaluated in a number of clinical trials and found to be safe and highly effective in clearing *P. falciparum* infections, even in areas with high CQ resistance.\(^2\)\(^3\)\(^4\) Thus, AQ is now considered as an alternative to CQ and S/P for the management of uncomplicated falciparum malaria in Africa.\(^4\) S/P and AQ became first- and second-line anti-malarials in Tanzania in 2001 because of high-level CQ resistance. When we conducted the study we are reporting here, SP resistance was already high in Magoda and Mpapayu villages, similar to other lowland areas of Muheza.\(^21\)\(^24\)\(^25\)\(^32\) Data from ongoing drug failure surveillance at the two villages have shown consistently better response with AQ in 14-day follow-up (M. M. Lemnge and others, unpublished data). For instance, the 2002 AQ efficacy study in the Magoda village showed good response in a 14-day follow-up period (ACR of 100%).

In our study of May 2003, an extended 28-day *in vivo* test\(^15\) to assess the efficacy of AQ in children with uncomplicated falciparum malaria, in the villages of Magoda and Mpapayu (with ITNs) and Mgome (without ITNs), was conducted. The significantly higher (\( P < 0.001 \)) prevalence of malarial parasites in Mgome (83.3%) compared with Magoda and Mpapayu villages (34.6%) seen during the screening process is most likely a direct impact of ITNs and mobile clinic operating thrice weekly in the latter villages. The impact of ITNs resulted in lower parasite densities in the ITN village, but this was not significant. Indeed, the low parasite densities found in the intervention villages was the main contributor for the exclusion of cases at recruitment. Notwithstanding this, our baseline data for the cohort children treated with AQ was comparable in terms of parasite density, anemia, age, and body temperature, all of which are known to influence treatment outcome.\(^5\)\(^33\)

PCR was used for parasite genotyping of the *msp1* and *msp2* genes and the molecular marker of chloroquine resistance, *Pfcrt*, in the study cohort. The efficacy data obtained revealed quick and sustained parasite clearance within 7 days of follow-up. Testing of all day 7 samples by nested *msp2* PCR confirmed they were all negative as seen by microscopy; indicating that in the first week after AQ treatment, there was an initial clearance of asexual parasites from the blood. Furthermore, based on the *msp1* and *msp2* PCR results, some of the LTF cases were indeed reinfections; confirming the importance of using PCR to distinguish recrudescences from reinfections.\(^28\) Taken together, these findings indicate that AQ is not that efficacious in the treatment of uncomplicated falciparum malaria despite the initial parasite clearance in both community settings. This initial resolution in both clinical and infection loads are of clinical significance in the management of malaria. However, usefulness of AQ beyond day 14 in areas of high malaria transmission is limited as evidenced by the eventual reduction in the initial excellent gain in both parasite clearance and mean PCV levels in both ITN and non-ITN villages. The lack of sustained gain in PCV levels beyond day 14 might be caused by the recrudescence and re-infecting parasites. The higher parasite clearance in the ITN compared with the non-ITN villages, after day 14, suggest a direct impact of ITNs in preventing reinfections that might curtail the development of resistance.\(^20\)

Recently, it has been reported that there is an association between mutant allele *Pfcrt* K76T and AQ resistance *in vivo* in southern Sudan, where CQ is still efficacious.\(^18\) In this study, the *Pfcrt* analysis showed that there was an extremely high frequency of *Pfcrt* baseline mutation and haplotype CVIET. High parasite clearance rate at day 7 suggests that

### Table 2

Treatment outcome for 54 children treated with AQ and successfully followed-up in a 28-day *in vivo* test in ITN and non-ITN villages in Muheza, northeastern Tanzania

<table>
<thead>
<tr>
<th>Treatment outcome</th>
<th>ITN (N = 28)</th>
<th>Non-ITN (N = 26)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early treatment failure</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Late treatment failure</td>
<td>17 (60.7%) uncorrected</td>
<td>23 (88.5%) uncorrected</td>
<td>0.02</td>
</tr>
<tr>
<td>Adequate clinical and parasitological response</td>
<td>6 (21.4%) PCR corrected</td>
<td>9 (34.6%) PCR corrected</td>
<td></td>
</tr>
</tbody>
</table>

| Adequate clinical and parasitological response | 11 (39.3%) | 3 (11.5%) |      |

Significantly lower rate of late treatment failure in ITN compared to non-ITN villages (60.7% vs 88.5%; \( \chi^2 = 5.405, P = 0.02 \)).

### Table 3

Mean packed cell volume (mean PCV, %, SD) in children treated with AQ in ITN and non-ITN villages in Muheza, northeastern Tanzania

<table>
<thead>
<tr>
<th>Follow-up day</th>
<th>ITN</th>
<th>Non-ITN</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV0</td>
<td>31.0 (4.4)</td>
<td>28</td>
<td>30.5 (4.7)</td>
</tr>
<tr>
<td>PCV14</td>
<td>33.4 (4.1)</td>
<td>27</td>
<td>33.5 (2.9)</td>
</tr>
<tr>
<td>PCV21</td>
<td>33.3 (4.6)</td>
<td>26</td>
<td>30.8 (6.7)</td>
</tr>
<tr>
<td>PCV28</td>
<td>32.6 (4.3)</td>
<td>25</td>
<td>32.0 (3.7)</td>
</tr>
</tbody>
</table>

Significant gain in PCV day 14 compared with baseline in both settings (ITN: \( t = 4.0959, P = 0.0004 \); non-ITN: \( t = 3.9403, P = 0.0006 \)).

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<td>26</td>
<td>30.8 (6.7)</td>
</tr>
<tr>
<td>PCV28</td>
<td>32.6 (4.3)</td>
<td>25</td>
<td>32.0 (3.7)</td>
</tr>
</tbody>
</table>

Significant gain in PCV day 14 compared with baseline in both settings (ITN: \( t = 4.0959, P = 0.0004 \); non-ITN: \( t = 3.9403, P = 0.0006 \)).

Slightly higher PCV on day 28 compared with baseline in ITN village but not significant (ITN: \( t = 1.986, P = 0.0566 \)).

No significant gain in PCV day 28 compared with baseline in the non-ITN village (\( t = 1.2678, P = 0.2175 \)).
AQ was initially efficacious, and cleared Pfcr7 mutated parasites. However, six samples were not the single CVIET haplotype before treatment, of which four were the recrudescent, and all resulted in single CVIET haplotype infections. Thus, selection of the single CVIET haplotype after AQ treatment is probably occurring. However, these limited data are insufficient to confirm this. The results indicate a high rate of reinfections, emphasizing the importance of PCR adjustment, especially in settings with high transmission intensity. AQ was found to be effective against CQ-resistant Pfcr7 mutant field isolates. Notwithstanding, the high frequency of the CVIET mutation haplotype in pretreatment samples suggests it could not be used as a predictor of AQ treatment failures.

In conclusion, AQ alone was found to be insufficiently effective in the 28-day follow-up period, despite the drug being of proven quality by both dissolution test and quantity. Its usefulness was limited to the first 2 weeks after treatment as previously observed in other studies. A similar study involving a larger sample size is recommended that can have sufficient power to assess the relationship between Pfcr7 codon 72–76 haplotypes and AQ treatment outcome in villages with and without ITNs. Use of fixed-dose artemisinin-based combination therapy like Coartem should now be considered in this area; indeed, this combination therapy (CT) is going to be the first-line anti-malarial in Tanzania starting in 2006. AQ plus artesunate (AQ/AS) is one of the loose CTS recently tested at some sentinel sites in Tanzania in an attempt to explore its performance. Preliminary data indicate that it is a promising alternative despite high reinfections in some areas (unpublished data). Ongoing studies done elsewhere with the AQ/AS fixed CT have shown its potential, and if used with ITNs, would be effective and relatively cheap (unpublished data).

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