Activities of amodiaquine, artesunate, and artesunate-amodiaquine against asexual- and -sexual stage parasites in falciparum malaria in children

Running title: antimalarial activities of artesunate and amodiaquine

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The activities of amodiaquine, artesunate and artesunate-amodiaquine against asexual- and sexual- stage parasites were evaluated in 360 Nigerian children with uncomplicated *Plasmodium falciparum* malaria randomized to the standard dose regimens of the three drugs/combination. Clinical recovery from illness occurred in all children. There were no significant differences in fever clearance times. Patients treated with artesunate or artesunate-amodiaquine had significantly shorter parasite clearance times (1.4 ± 0.5 or 1.4 ± 0.6 *versus* 3.2 ± 2.3 d, *P* = 0.0001) and lower gametocyte carriage rates (3.3 or 1.7 *versus* 11.7%, *P* = 0.001) than those treated with amodiaquine alone. Gametocytaemia was detected in 62 patients (11.7% before treatment and 5.6% after treatment). The pretreatment gametocyte sex ratio, which was female biased, increased significantly during the course of treatment with amodiaquine but not with artesunate and artesunate-amodiaquine. These results suggest that artesunate and artesunate-amodiaquine reduce gametocyte carriage and may reduce transmissibility in *P. falciparum* malaria by accelerating asexual clearance and influencing gametocyte sex ratio.

*Keywords: amodiaquine; artesunate; malaria; gametocyte sex ratio; children; Nigeria*
Multi-drug resistance in *Plasmodium falciparum* is an increasing public health problem in much of malaria endemic sub-Saharan Africa. As part of efforts to combat drug resistance, the World Health Organization (WHO) recommended the use of artemisinin-based combination antimalarial therapy (ACTs) in this and other endemic areas (20). Combination regimens which include artesunate clear parasitaemia rapidly and may reduce gametocyte transmissibility in areas of low transmission (11, 12).

In endemic areas of West Africa, up to 14-17% of the children with acute, uncomplicated falciparum malaria may carry gametocytes in their peripheral blood at presentation (16-18). These carriage rates may be increased following treatment with antimalarial monotherapy (17). In these areas, one of the most frequently used ACTs is artesunate-amodiaquine, the individual components of which are readily available, and readily used uncombined. However, the effects of these combination treatments on gametocyte carriage in areas of intense transmission in Africa have been less frequently evaluated. In addition, the effects of these combinations and those of their individual components on *P. falciparum* gametocyte sex ratio are unknown.

The aim of the present study was to evaluate the effects of adding artesunate to amodiaquine compared with amodiaquine or artesunate alone on asexual parasites, gametocyte carriage and sex ratio in children suffering from acute uncomplicated falciparum malaria.
Patients and Methods

Patients
The study was conducted in children aged < 11 years with acute uncomplicated *P. falciparum* malaria in Ibadan, a malaria endemic area (14), in southwestern Nigeria. Fully informed consent was obtained from the parents/guardians of each child. Inclusion criteria were: fever or history of fever in the 24-48 h preceding presentation, pure *P. falciparum* parasitaemia > 2000 asexual forms/µL, absence of other concomitant illness, no history of antimalarial use in the 2 weeks preceding presentation, and negative urine tests for antimalarial drugs (Dill-Glazko and lignin). Patients with severe malaria (19), severe malnutrition, serious underlying diseases (renal, cardiac, or hepatic), and known allergy to study drugs were excluded from the study. The study protocol was approved by the Ethics Committee of the Ministry of Health, Ibadan, Nigeria.

Drug Management
After clinical assessment, blood was obtained for haematocrit determination and for quantification of asexual and sexual parasitaemia. Patients were randomized to (i) three-day regimen of amodiaquine base at 10 m/kg daily (day 0-2), (ii) artesunate at 4mg/kg daily for 7 days (day 0-6), and (iii) and a 3-day combination of artesunate and amodiaquine at the doses given in (i) and (ii) above. All drugs were given orally and all patients waited for at least 3 h after to ensure the drug was not vomited. If it was, the patient was excluded from the study.

Oral paracetamol (acetaminophen) at 10-15 mg/kg 6 hourly was given for 12-24 h if body temperature was > 38°C. Patients were seen daily, at approximately the same time of the
day for the first five days (days 0-4) and then daily on days 7, 14, 21, 28 and when necessary on day 35 after treatment had begun. At each visit, patients were assessed clinically and thick and thin blood smears were obtained for quantification of parasitaemia.

The fever clearance time (FCT) was defined as the time taken for the body temperature to fall to below 37.5°C and remain below this value for > 48 h.

**Laboratory investigations**

Asexual parasite and gametocyte counts were measured daily for the first five days (days 0-4) and thereafter on days 7, 14, 21 and 28. Quantification in Giemsa-stained thick blood films was done against 500 leukocytes in the case of asexual parasitaemia, and against 1000 leukocytes in the case of gametocytes, and from this figure, the parasite density was calculated assuming a leukocyte count of 6000/µl of blood. Parasite clearance time (PCT) was the time interval from the start of antimalarial treatment until the asexual parasite count fell below the detectable levels in a peripheral blood smear. Capillary blood, collected before and during follow-up, was used to measure packed cell volume (PCV). PCVs were measured using a microhaematocrit tube and microcentrifuge (Hawksley, Lancing, UK). Routine haematocrit was done on days 0, 3, 7, 14, 21 and 28.

**Determination of gametocyte sex ratio**

Gametocyte sex determination was based on the following criteria (4, 13): males (microgametocytes) are smaller than females (macrogametocytes), the nucleus is larger in
males than females, the ends of the cells are rounder in males and angular in females, with Giemsa the cytoplasm stains purple in males and deep blue in females, and the granules of malaria pigment are centrally located females and more widely scattered in males. The sex ratio was defined as the proportion of gametocytes in peripheral blood that were male (10). Gametocytes were sexed if the gametocyte density was \( \geq 15/\mu l \) blood.

For each patient, gametocyte densities were plotted against time. The areas under the curve of gametocytaemia versus time (AUC\textsubscript{gm}) were determined by a non-compartmental method using a computer programme Turbo Ken (Clinical Pharmacology Group, University of Southampton, UK, through the courtesy of Prof. A.G. Renwick) as previously described (16). Briefly AUC\textsubscript{gm} were obtained, using linear trapezoidal rule, from time zero (0 h, day 0) to time of gametocyte clearance, or if there was no clearance, till 672 h (day 28). The final gametocytaemia at time of clearance was assumed to be 0.001 sexual forms (sf)/\( \mu l \) blood (a level assumed to be below microscopic detection).

Data analysis. Data were analyzed using version 6 of the Epi-Info software (2), and the statistical programme SPSS for Windows version 10.01 (3). Variables considered in the analysis were related to the densities of \textit{P. falciparum} gametocytes and trophozoites. Proportions were compared by calculating \( \chi^2 \) with Yates’ correction or by Fisher exact or by Mantel Haenszel tests. Normally distributed, continuous data were compared by Student’s t-tests and analysis of variance (ANOVA). Data not conforming to a normal distribution were compared by the Mann-Whitney U-tests and the Kruskal-Wallis tests.
(or by Wilcoxon ranked sum test). Kaplan-Meier plots are also presented to compare gametocyte carriage rates following treatment in those who were gametocytaemic at presentation. Differences in survival time were assessed by inspection of Kaplan-Meier curves and log-rank tests. All tests of significance were two-tailed. P-values of \( \leq 0.05 \) were taken to indicate significant differences. Data were (double)-entered serially using the patients codes and were only analyzed at the end of the study.
Results

Patients

Between November 2005 and October 2006, 360 children (178 males, 182 females) with *P. falciparum* malaria, aged between 0.5-12 years (mean ± standard deviation [SD] = 6.3 ± 2.6 years) were enrolled. One hundred and twenty patients each were randomized to each of the three treatment arms (Table 1). There were no significant differences in enrolment characteristics between all the 3 treatment groups, but children enrolled in artesunate-amodiaquine group weighed significantly more than those enrolled in the amodiaquine and artesunate alone groups.

Clinical responses

All children responded promptly to treatment, and none developed severe malaria. The overall median (range) FCT was 1.0 (1-3 d), and was not significantly different between the 3 treatment groups. None of the studied children had significant adverse effects as monitored by clinical symptoms (data not shown) but 3 and 4 children treated with amodiaquine, and artesunate-amodiaquine, respectively reported pruritus which interfered with sleep.

Parasitological responses

The overall mean PCT ± SD was 1.9 ± 1.6 d and was significantly shorter in the artesunate (1.4 ± 0.5 d) and artesunate-amodiaquine (1.4 ± 0.6 d) groups than in the amodiaquine alone (3.0 ± 2.3 d) group (P = 0.0001) (Table 1). There was no significant difference in mean PCT between the artesunate and artesunate-amodiaquine groups (P =
The parasite reduction ratios (PRR) (parasite count at enrolment/parasite count at day 2) were significantly higher in the artesunate alone (median, 99.4; range 500 - 666,000) and artesunate-amodiaquine (median, 74; range 3 - 827,200) groups than in the amodiaquine alone (median, 52.6; range 1-1,668,000) group (P = 0.03) (Table 1). There was no significant difference in PRR between the artesunate and artesunate-amodiaquine groups (P = 0.119).

Clinical course
Overall 351 (97.5%) of the enrolled children completed at least 21 days of follow-up (Table 2). Of these 351, 25 had subsequent reappearance of *P. falciparum*. Children treated with artesunate had a significantly higher rate of reappearance (20 of 111) than children treated with amodiaquine alone (4 of 120) and artesunate-amodiaquine (2 of 120) ($\chi^2 = 29.7$, df = 2, $P = 0.0000003$). There was no significant difference in the rate of reappearance between the amodiaquine alone and the artesunate-amodiaquine groups ($P = 0.36$). Overall, the polymerase chain reaction (PCR)-uncorrected cure rate was 92.8%, and it ranged from 81.9% in the artesunate group, to 96.7% in the amodiaquine group, to 99.2% in the artesunate-amodiaquine group. Overall, following clearance of parasitaemia, the interval to reappearance was $17.6 \pm 4.8$ d (mean $\pm$ SD) and was similar in the amodiaquine alone (14.0 $\pm$ 0.0 d) and artesunate alone (18.6 $\pm$ 4.3 d) alone groups. In artesunate treated children, the parasitaemia at enrollment in those in whom parasitaemia reappeared within two weeks of treatment (geometric mean = 42482, range 4211-42857, $n = 2$) was similar to parasitaemia in those in whom parasitaemia appeared
between 16-21 days (geometric mean = 48791, range 1300-597600, n = 10) (P = 0.55). Gametocytes were detected at the time of reappearance of parasitaemia in 1 child each from the artesunate and amodiaquine alone groups but not in the artesunate-amodiaquine group.

Gametocytaemia

Gametocytes were detected in peripheral blood in 62 children (17.2%) from all three groups (in 42 children before treatment and in 20 children after initiation of treatment) (Table 3). The overall detection rate at enrolment was 11.7% (n= 42) and it was not significantly different between the 3 treatment groups (P = 0.72). Following treatment, the emergence of gametocyte was significantly less frequent in the artesunate and artesunate-amodiaquine groups than in the amodiaquine alone group (4 and 2 children, respectively versus 14 children) (P = 0.001). The gametocyte detection rates after treatment with artesunate and artesunate-amodiaquine were not significantly different (P = 0.6).

Duration of gametocyte carriage in children with gametocytaemia at enrolment

The probability of a mosquito infectivity following a blood meal is related to gametocyte density and the duration of carriage by the host. Figure 2 is a Kaplan-Meier plot (survival curve) of the cumulative probability of remaining gametocyte free following treatment with amodiaquine, artesunate, and artesunate-amodiaquine in children who
were gametocytaemic at presentation. This probability was highest with artesunate alone and lowest with amodiaquine alone. Thus, compared with amodiaquine alone, children treated with artesunate alone had a significantly higher propensity to remain gametocyte free (Log rank statistic = 4.97, P = 0.02). Compared with artesunate-amodiaquine-treated children, the probability of remaining gametocyte free in those treated with artesunate alone was not significantly different (Log rank statistic = 3.16, P = 0.07). Comparison of this probability between children treated with amodiaquine alone and those treated with artesunate-amodiaquine was also not significantly different (Log rank statistic = 0.02, P = 0.88).

**Temporal changes in gametocyte sex ratio**

In the 42 children who were gametocytaemic at presentation, a total of 1440, 1068, 1152, 1554, 264, 1254, 276, 30 and 582 gametocytes were counted on days 0, 1, 2, 3, 4, 7, 14, 21, and 28, respectively. Of these, 1158, 858, 954, 1494, 240, 1152, 270, 30 and 420, gametocytes could be sexed on days 0, 1, 2, 3, 4, 7, 14, 21 and 28, respectively. The corresponding number of patients in whom the gametocytes were counted was 42, 24, 23, 32, 8, 25, 11, 2, and 2, respectively.

A male biased sex ratio may increase the chance of transmission. Following treatment, in amodiaquine-treated children, gametocyte sex ratio was significantly more male biased on days 3 and 7 compared with those treated with artesunate or artesunate-amodiaquine (Table 4). Following treatment with amodiaquine, gametocyte sex ratio increased
significantly over the course of the infection and up to 14 days after start of treatment in children who were gametocytaemic at enrolment (Figure 2 and Table 4): 24% of the gametocytes were male at day 0, 60% at day 3, and 63% at day 7 \( (\chi^2 = 4.5, P = 0.03) \). By contrast, the was no significant increase in gametocyte sex ratio in children treated artesunate alone: 11% of the gametocytes were male at day 0, 16% at day 3, and 0% at day 7 \( (\chi^2 = 1.1, P = 0.27) \) and artesunate-amodiaquine: 6% of the gametocytes were male at day 0, 9% at day 3, and 7% at day 7 \( (\chi^2 = 0.29, P = 0.58) \) (Table 4). The increase in sex ratio in amodiaquine-treated children was associated with increase in mean gametocyte density, and an initial reduction followed by a rise in packed cell volume. By contrast, minimal increase in sex ratio in children treated with artesunate and artesunate-amodiaquine was accompanied by a decrease in gametocyte density, and an initial reduction followed by a rise in packed cell volume (Figure 2).

\[ AUC_{gm} \text{ were determined only in patients who carried gametocytes at presentation, that is, the 16, 12, and 14 patients who were treated with amodiaquine, artesunate, and artesunate-amodiaquine, respectively. The mean of } AUC_{gm} \text{ were } 492 \pm 362 \text{ [mean } + \text{ sem], range } 4.4 - 1449.6 \text{ sexual form}/\mu\text{l.d, 126.7} \pm 32.3, \text{ range } 4.4 - 111.8 \text{ sexual form}/\mu\text{l.d, and } 167.5 \pm 78.8, \text{ range } 4.4 - 295.1 \text{ sexual form}/\mu\text{l.d in amodiaquine, artesunate, and artesunate-amodiaquine treatment groups, respectively. The mean } AUC_{gm} \text{ for amodiaquine was four and three folds higher than those for artesunate and artesunate-amodiaquine, respectively.} \]
Discussion

For control programmes, the ideal antimalarial or combination antimalarial should rapidly clear asexual parasitaemia and its associated clinical symptoms and signs within the shortest possible time, in addition to preventing transmissibility and reducing the chances of development of drug resistance. We have documented the effects of amodiaquine, artesunate or its combination with amodiaquine on asexual- and sexual-stage parasites in a cohort of children with uncomplicated falciparum malaria in an endemic area. The study was based on a follow-up period of 28 days. The results showed that amodiaquine, artesunate or its combination with amodiaquine rapidly cleared asexual parasitaemia without producing undue deleterious effects. Artesunate or its combination with amodiaquine were more rapidly acting than amodiaquine alone as assessed by the parasite clearance times and parasite reduction ratios. These findings support previous reports from the same region of Africa (1). Although the cure rates from the three treatment groups were similar on day 28, a longer period of follow up in addition to molecular genotyping to distinguish recrudescence from reinfection, would have made it possible to determine the true cure rates.

In this study, parasites reappeared from peripheral blood in 7% of the children by 21 days of observation period. The rate of reappearance was significantly higher with artesunate alone than with its combination with amodiaquine or amodiaquine alone. A striking feature of the patients with reappearance of parasitaemia in the artesunate treated children was that reappearance within two weeks was not associated with higher parasite burden at enrolment compared with the parasite burden at enrolment in children in whom there
was reappearance after two weeks. Since recrudescence after artesunate monotherapy is often associated with heavy parasite burden at enrolment (7), we have no clear explanation for our observation. Although parasite genotyping was not done before and after reappearance, it is likely that the early reappearance was due to recrudescence and the late reappearance to re-infection. In this regard, molecular genotyping would have been of great value in distinguishing recrudescence from reinfection.

Overall, children treated with artesunate or its combination with amodiaquine had significantly lower gametocyte carriage rates than those treated with amodiaquine alone. In addition, the cumulative probability of remaining gametocyte free in those who were gametocytaemic at enrolment was highest in those treated with artesunate alone. The latter would suggest that the artemisinin derivatives have significant effects on gametocyte clearance as previously suggested by others (12). However, we have no explanation for the finding that artesunate-amodiaquine was not better than amodiaquine alone, although the AUC$_{gm}$ from amodiaquine was three folds higher than AUC$_{gm}$ from artesunate-amodiaquine. The mechanisms by which the artemisinin derivatives clear gametocyttaemia have not been full elucidated; our results suggest artesunate may not rapidly clear mature gametocytes from the peripheral circulation, rather the drug, by rapidly clearing asexual parasites prevent the progression of committed asexual parasites to gametocytes.

Our study evaluated the effects of treatment with amodiaquine, artesunate or its combination with amodiaquine on gametocyte sex ratio in children who were
gametocytaemic at presentation. To our knowledge, this is the first designated study of the effects of these drugs on gametocyte sex ratio in African children. Overall, the gametocyte sex ratio was female-biased at enrolment in keeping with that of natural population (15). In those treated with amodiaquine gametocyte sex ratio increased over the course of treatment in the presence of increased gametocyte density and despite clearance of asexual parasitaemia by day 3 of treatment, suggesting that the drug may have some effect on gametocyte sex ratio. However, the presence of anaemia, a factor that enhances gametocyte maleness (8, 9) in these children suggests that there may be other contributory factors to the maleness observed. This interaction needs further elucidation. By contrast, despite the presence of anaemia, and decreased gametocyte density, factors which promote gametocyte maleness, the gametocyte sex ratio was still female-biased in the children treated with artesunate or its combination with amodiaquine. The reasons for our observations are unclear. The artemisinin derivatives, compared with the other antimalarial drugs, for example, quinine, are less likely to provoke anaemia when asexual parasites are eliminated from the infected red blood cells (5). In addition, rapid clearance of asexual parasites by artesunate may have blunted the cues for gametocyte maleness which normally follow the course of infection (8). An unexplored possibility for the artesunate-related female biased gametocyte sex ratio is that the artemisinin derivatives may selectively kill male gametocytes. Whatever their modes of influence on the gametocyte sex ratio, carefully designed studies are urgently required to delineate the influence of the artemisinin derivatives on the sex ratio of Plasmodium spp, for example, laboratory mosquitoes feeding studies would be necessary to assess transmissibility. Thus, in addition to reducing gametocyte carriage and
infectiousness to mosquitoes (6), artesunate may reduce transmissibility possibly by influencing gametocyte sex ratio following treatment.

Acknowledgements

We are grateful to our clinic staff especially Moji Amao and Adeola Alabi for assistance with running the study.
References


The mechanism of parasite clearance after antimalarial treatment of *Plasmodium falciparum* malaria. J. Infect. Dis. 182: 629-633


Table 1. Demographic data and immediate therapeutic response for children with *P. falciparum* malaria

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values* for the following treatment groups</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amodiaquine</td>
<td>Artesunate</td>
</tr>
<tr>
<td>No. of patients</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Male/Female</td>
<td>50/70</td>
<td>64/56</td>
</tr>
<tr>
<td>Age (year)</td>
<td>6.7 ± 3.2</td>
<td>5.9 ± 2.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>18.6 ± 6.6</td>
<td>16.5 ± 5.1</td>
</tr>
<tr>
<td>Duration of illness (d)</td>
<td>2.9 ± 1.1</td>
<td>2.7 ± 0.8</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>38.0 ± 1.2</td>
<td>38.1 ± 1.0</td>
</tr>
<tr>
<td>Parasite count (/µL)</td>
<td>48045</td>
<td>47627</td>
</tr>
<tr>
<td>geometric mean</td>
<td>2014 – 1668000</td>
<td>2052 – 662000</td>
</tr>
<tr>
<td>parasite clearance time (d)</td>
<td>1.0 ± 0.3</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Parasite clearance time (d)</td>
<td>3.0 ± 2.3</td>
<td>1.4 ± 0.5</td>
</tr>
<tr>
<td>PRR (D2)**</td>
<td>52.6</td>
<td>99.4</td>
</tr>
<tr>
<td>(x 10^3)</td>
<td>0.001 – 1668.0</td>
<td>0.5 - 660</td>
</tr>
</tbody>
</table>

*Data are shown as mean ± SD or median (range), parasitaemia is geometric mean

** Parasite reduction ratio at Day 2
Table 2. Clinical outcome and time to reappearance of parasitaemia for children monitored up to 21 days or more after starting treatment

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No (%) of patients with</th>
<th>Completed follow-up</th>
<th>Subsequent appearance of <em>P. falciparum</em></th>
<th>Time to reappearance (median)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amodiaquine</td>
<td>120 (100)</td>
<td>4</td>
<td></td>
<td>14 (14-21) d+</td>
</tr>
<tr>
<td>Artesunate</td>
<td>111 (92.5)</td>
<td>12</td>
<td></td>
<td>21 (7-21) d</td>
</tr>
<tr>
<td>Artesunate-amodiaquine</td>
<td>120 (100)</td>
<td>1</td>
<td></td>
<td>9 d</td>
</tr>
<tr>
<td>Total</td>
<td>351 (97.5)</td>
<td>17</td>
<td>+ range</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 3. Gametocyte carriage in children with *P falciparum* malaria before and after treatment with amodiaquine, artesunate, and artesunate-amodiaquine

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No (%) of patients with gametocyte appearance</th>
<th>At enrolment</th>
<th>After enrolment</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amodiaquine (n = 120)</td>
<td></td>
<td>16 (13.3)</td>
<td>14 (11.7)</td>
<td>30</td>
</tr>
<tr>
<td>Artesunate (n = 120)</td>
<td></td>
<td>12 (10)</td>
<td>4 (3.3)</td>
<td>16</td>
</tr>
<tr>
<td>Artesunate-amodiaquine (n= 120)</td>
<td></td>
<td>14 (11.7)</td>
<td>2 (1.7)</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>42 (11.7)</td>
<td>20 (5.6)</td>
<td>62</td>
</tr>
</tbody>
</table>
Table 4. Variations in mean gametocyte sex ratio with time following treatment with amodiaquine, artesunate, and artesunate-amodiaquine

<table>
<thead>
<tr>
<th>Time of follow up (d)</th>
<th>Gametocyte sex ratio for the following treatment groups:</th>
<th>Gametocyte sex ratio for the following treatment groups:</th>
<th>Gametocyte sex ratio for the following treatment groups:</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amodiaquine</td>
<td>Artesunate</td>
<td>Artesunate-amodiaquine</td>
<td></td>
</tr>
</tbody>
</table>
| 0                    | \(0.24 \pm 0.20\)  
\(0.00 - 0.50^*\) | \(0.11 \pm 0.11\)  
\(0.0 - 0.23\) | \(0.06 \pm 0.08\)  
\(0.0 - 0.17\) | 0.16 |
| 1                    | \(0.37 \pm 0.30\)  
\(0.00 - 1.00\)  | \(0.13 \pm 0.16\)  
\(0.0 - 0.4\) | \(0.09 \pm 0.07\)  
\(0.0 - 0.17\) | 0.15 |
| 2                    | \(0.29 \pm 0.18\)  
\(0.00 - 0.5\)  | \(0.19 \pm 0.19\)  
\(0.0 - 0.5\) | \(0.04 \pm 0.08\)  
\(0.0 - 0.17\) | 0.13 |
| 3                    | \(0.60 \pm 0.21\)  
\(0.38 - 1.0\)  | \(0.16 \pm 0.15\)  
\(0.0 - 0.33\) | \(0.09 \pm 0.16\)  
\(0.0 - 0.29\) | 0.003 |
| 7                    | \(0.63 \pm 0.26\)  
\(0.33 - 1.0\)  | -                                              | \(0.07 \pm 0.09\)  
\(0.0 - 0.14\) | 0.05 |

Values are given as mean \(\pm\) sd, * range
Figure 1. Kaplan-Meier plot (survival curve) of cumulative probability of remaining gametocyte free, following treatment with amodiaquine (solid line), artemunate (thick broken line) and amodiaquine-artesunate (thin broken line), in children who were gametocytaemic at enrolment.
Figure 2. Variations in the packed cell volume, density of gametocytes and gametocyte sex ratio over the course of treatment of malaria infections with amodiaquine (solid line), artesunate (thick broken line) and amodiaquine-artesunate (thin broken line) in children with acute *Plasmodium falciparum* infection.