Moderate Effect of Artemisinin-Based Combination Therapy on Transmission of *Plasmodium falciparum*

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**Background.** Artemisinin-based combination therapy (ACT) reduces microscopically confirmed gametocytemia and mosquito infection. However, molecular techniques have recently revealed high prevalences of submicroscopic gametocytemia. Our objective here was to determine the effect of sulfadoxine-pyrimethamine (SP) monotherapy and treatment with SP plus amodiaquine (AQ), SP plus artesunate (AS), and artemether-lumefantrine (AL; Coartem) on submicroscopic gametocytemia and infectiousness.

**Methods.** Kenyan children (6 months–10 years of age) were randomized to 4 treatment arms. Gametocytemia was determined by both microscopy and Pfs25 RNA–based quantitative nucleic acid sequence–based amplification (Pfs25 QT-NASBA). Transmission was determined by membrane-feeding assays.

**Results.** Gametocyte prevalence, as determined by Pfs25 QT-NASBA, was 89.4% (219/245) at enrollment and decreased after treatment with SP plus AS, SP plus AQ, and AL. Transmission was determined by membrane-feeding assays. Gametocyte prevalence, as determined by Pfs25 QT-NASBA, was 89.4% (219/245) at enrollment and decreased after treatment with SP plus AS, SP plus AQ, and AL. Membrane-feeding assays for a group of randomly selected children revealed that the proportion of infectious children was as much as 4-fold higher than expected when based on microscopy. ACT did not significantly reduce the proportion of infectious children but did reduce the proportion of infected mosquitoes.

**Conclusions.** Submicroscopic gametocytemia is common after treatment and contributes considerably to mosquito infection. Our findings should be interpreted in the context of transmission intensity, but the effect of ACT on malaria transmission appears to be moderate and restricted to the duration of gametocyte carriage and the proportion of mosquitoes that are infected by carriers.

Antimalarial drug treatment targets the asexual blood stages of *Plasmodium falciparum*, which are responsible for clinical disease and death. Sexual-stage parasites—that is, gametocytes—do not cause clinical disease, and they can also be found in the circulation of infected persons and can infect mosquitoes taking a blood meal. After fertilization and further sporogonic development, infectious parasites appear in the mosquito salivary gland. With each subsequent blood meal, parasites are transmitted to a new person, resulting in the spread of malaria among the human population. The majority of the currently used antimalarial drugs do not kill gametocytes, and the effects of drugs on these stages are often ignored during the development of antimalarial drugs and policies.

The resistance of *P. falciparum* to such antimalarial drugs as chloroquine and sulfadoxine-pyrimethamine (SP) is rapidly increasing in many areas of sub-Saharan Africa. This has forced many African countries to abandon monotherapy with these drugs as first-line antimalarial treatment [1]. Because drug resistance is less likely to develop when combination therapy is used [2, 3], there is increasing acceptance that it, rather than monotherapy, is the way forward, with artemisinin being one of the preferred components [1, 2, 4]. Compared with monotherapy, artemisinin-based combination therapy (ACT) provides higher clinical efficacy [5, 6] in the absence of drug resistance. An additional advantage of ACT is its effect on sexual stages—it lowers...
the prevalence and density of posttreatment gametocytemia [5, 7, 8] and, subsequently, the prevalence of mosquito infection [7, 9, 10]. ACT may reduce malaria transmission at the population level [8, 11], and the specific reduction in the transmission of resistant strains [8, 11, 12] further suggests that ACT may counteract the spread of drug resistance.

In contrast to asexual parasites, gametocytes often circulate at such low densities that they may not be detected by standard microscopy [13–15]. Indeed, Pfs25 RNA–based quantitative nucleic acid sequence–based amplification (Pfs25 QT-NASBA) [16] recently revealed that a large proportion of children with symptomatic malaria had submicroscopic gametocytemia [17].

Our objective here was to determine, in Kenyan children treated for uncomplicated malaria, the clinical efficacy of drug regimens that are currently in use or are being considered for inclusion in national guidelines in different African countries; gametocyte prevalence by Pfs25 QT-NASBA [16]; and posttreatment infectiousness to *Anopheles gambiae* mosquitoes. The regimens analyzed were SP monotherapy and 3-day courses of SP plus amodiaquine (AQ), SP plus artesunate (AS), and artemether-lumefantrine (AL) [1, 6].

**SUBJECTS, MATERIALS, AND METHODS**

The present study was conducted from October to December in 2003 and 2004 in Mbita, a rural village on the shores of Lake Victoria in Suba District, western Kenya. The main malaria vectors in the area are *A. gambiae*, *A. funestus*, and *A. arabiensis*. Malaria transmission is high and perennial, with parasite prevalences in the human population ranging from 24.4% to 99.0% [18]. Generally, the rainfall pattern is bimodal, with a long rainy season between March and May and a short rainy season between October and December. The study protocol (SSC 791) was approved by the Scientific Steering Committee and the Ethical Review Committee of the Kenya Medical Research Institute. Written, informed consent was obtained from a parent or guardian of the participating children.

**Study population and treatment.** Subjects were recruited at the clinic of the International Centre of Insect Physiology and Ecology (ICIPE) Mbita Point Field Station and came from an area of ~10 km around the ICIPE compound. Children 6 months–10 years of age with either a temperature of >37.5°C or a history of fever and with *P. falciparum* mono-infection at an asexual parasite density of ≥500 parasites/μL were eligible for recruitment. Exclusion criteria were the inability to take drugs orally, known hypersensitivity to any of the drugs given, reported treatment with antimalarial chemotherapy during the previous 2 weeks, evidence of chronic disease or of an acute infection other than with a malarial parasite, residence outside of the study area, and signs of severe malaria. Children were randomly allocated to receive 1 of the 4 following treatment regimens: (1) SP monotherapy, which consisted of 25 mg/kg sulfadoxine and 1.25 mg/kg pyrimethamine as a single dose (Fansidar; Roche) plus placebo once daily for 3 days; (2) SP plus 4 mg/kg AS (Arsumax; Sanofi) once daily for 5 days; (3) SP plus 10 mg/kg AQ (Camoquine; Pfizer) once daily for 3 days; and (4) AL (Coartem; Novartis Pharma), administered as half a tablet (20 mg of artemether and 120 mg of lumefantrine) per 5 kg of body weight in a 6-dose regimen (at enrollment and 8, 20, 32, 44, and 56 h [±90 min] after the initiation of treatment).

Children were randomized to the SP monotherapy and the SP plus AS arms in 2003 and to the SP monotherapy, SP plus AS, SP plus AQ, and AL arms in 2004. The sizes of the arms were not equal, for several reasons. At the time of the study, AL was not recommended for children who weighed <10 kg. In addition, the 6-dose AL regimen required treatment to be administered at the child’s home. Therefore, only those children who weighed ≥10 kg and resided <5 km from the clinic (to allow supervised treatment at home) were eligible for randomization to the AL arm. This resulted in a smaller number of children in this treatment arm.

The numbers of children in the SP monotherapy arm and the SP plus AS arm were highest because the relevance of the random-feeding assays became evident during the latter part of data collection in 2004. For these assays, an additional number of children had to be randomized to the SP monotherapy and the SP plus AS arms. Therefore, to supplement the number of children who received these regimens in 2003 (*n* = 245), 81 children were randomized to the SP monotherapy and the SP plus AS arms at a 1:2 ratio in 2004. This ratio was chosen to facilitate comparison between the SP plus AS arm and the AL arm, for which parasitological differences were expected to be smallest.

For SP monotherapy and treatment with SP plus AQ and SP plus AS, treatment was administered by staff at the recruitment clinic; for treatment with AL, the first dose was administered by staff at the recruitment clinic, and subsequent doses were administered by field assistants at the child’s home. All medication was given with a local fatty food (mandazi), to facilitate absorption. Each child was observed for 20 min after administration of treatment, and a replacement dose was given if vomiting occurred. Repeated vomiting led to exclusion from the study; paracetamol (10 mg/kg) was given until symptoms had subsided.

Children were encouraged to return to the recruitment clinic on days 1, 2, 3, 7, 14, and 28 after initiation of treatment and at any time the child became ill. Field assistants visited the homes of children who failed to appear at the clinic, to collect additional samples. Other than those administering medication, all staff engaged in the trial were blinded to the treatment arm of each child.

**Microscopy and Pfs25 QT-NASBA.** Blood smears were
stained with 10% Giemsa for 10 min and then screened for asexual parasites and gametocytes at enrollment and on days 3, 7, 14, and 28. Slides were considered to be negative if no parasites were observed in 100 microscopic fields, resulting in a sensitivity of ~5 gametocytes/μL of blood. Asexual parasites and gametocytes were counted against 200 and 500 white blood cells (WBCs), respectively, and the counts were converted to parasites per microliter on the assumption of a density of 8000 WBCs/μL. For quality control, 10% of the slides were reread. Parasite detection by Pfs25 QT-NASBA was done as described elsewhere [16, 19], using a NucliSens EasyQ analyser (bioMérieux) as described elsewhere for Pfs25 mRNA [17]. Nucleic acid was extracted from 50-μL blood samples as described by Boom et al. [20]. The Pfs25 QT-NASBA technique is gametocyte specific and has a detection limit of 20–100 gametocytes/μL. NucliSens Basic kits were used for amplification, in accordance with the manufacturer’s instructions. A standard dilution series of mature, in vitro–cultured NF54 gametocytes [21] was included in each run. Detection by Pfs25 QT-NASBA was done for a random selection of children from each treatment arm and for all of the samples included in the membrane-feeding assays.

**Mosquito membrane feeding and dissection.** Membrane-feeding assays were conducted on day 14, when the gametocytes that develop after treatment are expected to be mature [22]. Children >2 years of age whose parent or guardian gave specific consent for the procedure were eligible for the membrane-feeding assays. Two groups of subjects were selected: (1) children with microscopically confirmed gametocytemia on day 7, which has been shown to be the time when the peak prevalence after treatment occurs [7, 23]; and (2) a random selection of children from each treatment arm (including children with and without gametocytemia by both microscopy and Pfs25 QT-NASBA).

For all membrane-feeding assays, 3-mL venous blood samples were obtained and fed to ~150 locally reared [24] 4–5-day-old female *A. gambiae* sensu stricto mosquitoes via an artificial membrane attached to a water-jacketed glass feeder maintained at 37°C. After 10–15 min, fully fed mosquitoes were selected and kept on glucose for 7 days at 27°C–29°C, at which time midguts were dissected in 2% mercurochrome. Midguts were examined microscopically for oocysts, with a second microscopist confirming their presence if observed. Assay results were considered to be valid if a minimum of 20 mosquitoes were dissected. For the randomly selected children, exactly 30 mosquitoes were dissected per child.

**Molecular genotyping.** For DNA analyses, blood was collected as dry blood on Whatman glass microfiber filter papers. DNA was extracted using a chelex-based method. Alleles of the polymorphic locus *msp2* were compared between pretreatment and posttreatment parasite isolates by polymerase chain reaction, as described elsewhere [10]. The procedure of Cattamanchi et al. [25] was followed in that indeterminate samples for which a majority of novel bands appeared for the posttreatment infection were scored as new infections.

**Statistical analyses.** Treatment outcome was classified as

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**Figure 1.** Study profile. AL, artemether-lumefantrine; APD, asexual parasite density; AQ, amodiaquine; AS, artesunate; SP, sulfadoxine-pyrimethamine.
were used, to allow for autocorrelation. Regression coefficients logistic regression models and generalized estimating equations analyses of gametocyte prevalence during follow-up, multiple compared using the Kruskal-Wallis or Wilcoxon rank-sum test. For test. Variables that were not normally distributed were com-

mean; IQR, interquartile range; SP, sulfadoxine-pyrimethamine.

Fever (\(\geq 37.5^\circ C\)) were directly determined for the randomly infected mosquitoes were directly determined for the randomly selected children. Relative risks and 95% confidence intervals infected at least 1 mosquito; this method was

were not significantly different among the 4 treatment arms (table 1). The median age (\(P = .03\)), weight (\(P = .03\)), and age-associated hemoglobin level (\(P = .01\)) were slightly higher for the children in the AL arm than for the children in the other arms, because only subjects who weighed \(\geq 10\) kg were included in the AL arm. This imbalance was accounted for in the statistical analyses and did not confound any of the observed relationships. During follow-up, SP monotherapy produced an adequate clinical response in 44.1% (63/143) of the children, and early treatment failure was observed in 9.8% (14/143) of the children in this arm (table 2). The combination regimens resulted in fewer treatment failures, and adequate clinical response was observed in 9.8% (14/143) of the children in this arm (table 2). The combination regimens resulted in fewer treatment failures, and adequate clinical response was observed in 9.8% (14/143) of the children in this arm (table 2).

Clinical efficacy. A total of 528 children were randomized to the 4 treatment arms (figure 1). Two children died within 1 day of enrollment, as a result of factors other than malaria. Of the remaining children, 6.3% (33/526) were lost for evaluation during the 28-day follow-up period. Asexual parasite density, gametocyte prevalence, and frequency of fever at enrollment were not significantly different among the 4 treatment arms (table 1).

The results of the membrane-feeding assays were analyzed on 2 outcomes. The percentage of the population that was infectious was estimated by multiplying the percentage of children with gametocytemia on day 7 and the percentage of these children who infected at least 1 mosquito; this method was used because it is in accordance with that of previous studies of malaria transmission after antimalarial drug treatment [7, 9, 10] and, hence, allowed comparison of results. In addition, the percentage of infectious children and the percentage of infected mosquitoes were directly determined for the randomly selected children. Relative risks and 95% confidence intervals were calculated using the SP monotherapy arm as the reference group.

**RESULTS**

Clinical efficacy. A total of 528 children were randomized to the 4 treatment arms (figure 1). Two children died within 1 day of enrollment, as a result of factors other than malaria. Of the remaining children, 6.3% (33/526) were lost for evaluation during the 28-day follow-up period. Asexual parasite density, gametocyte prevalence, and frequency of fever at enrollment were not significantly different among the 4 treatment arms (table 1). The median age (\(P = .03\)), weight (\(P = .03\)), and age-associated hemoglobin level (\(P = .01\)) were slightly higher for the children in the AL arm than for the children in the other arms, because only subjects who weighed \(\geq 10\) kg were included in the AL arm. This imbalance was accounted for in the statistical analyses and did not confound any of the observed relationships. During follow-up, SP monotherapy produced an adequate clinical response in 44.1% (63/143) of the children, and early treatment failure was observed in 9.8% (14/143) of the children in this arm (table 2). The combination regimens resulted in fewer treatment failures, and adequate clinical response was observed in 9.8% (14/143) of the children in this arm (table 2). The combination regimens resulted in fewer treatment failures, and adequate clinical response was observed in 9.8% (14/143) of the children in this arm (table 2). The combination regimens resulted in fewer treatment failures, and adequate clinical response was observed in 9.8% (14/143) of the children in this arm (table 2).

Gametocyte prevalence by Pfs25 QT-NASBA. During fol-

Table 1. Characteristics of the study population at enrollment, by treatment arm.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SP monotherapy (n = 152)</th>
<th>SP plus AQ (n = 127)</th>
<th>SP plus AS (n = 174)</th>
<th>AL (n = 75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (IQR), years</td>
<td>3.5 (2.0–5.3)</td>
<td>2.6 (1.4–4.9)</td>
<td>3.1 (1.8–5.1)</td>
<td>4.1 (2.0–6.4)</td>
</tr>
<tr>
<td>Sex, male</td>
<td>50.7 (77/152)</td>
<td>52.8 (67/127)</td>
<td>49.4 (86/174)</td>
<td>53.3 (40/75)</td>
</tr>
<tr>
<td>Weight, median (IQR), kg</td>
<td>14.0 (11.0–18.4)</td>
<td>13.0 (10.0–16.1)</td>
<td>13.5 (10.0–18.0)</td>
<td>16.0 (12.0–20.2)</td>
</tr>
<tr>
<td>Fever ((\geq 37.5^\circ C))</td>
<td>66.2 (100/151)</td>
<td>53.5 (68/127)</td>
<td>64.7 (112/173)</td>
<td>54.7 (41/75)</td>
</tr>
<tr>
<td>Hemoglobin level, median (IQR), g/dL</td>
<td>9.4 (7.9–10.7)</td>
<td>9.3 (8.0–10.7)</td>
<td>9.4 (7.8–10.6)</td>
<td>10.4 (8.6–11.8)</td>
</tr>
<tr>
<td>Gametocytemia</td>
<td>20.9 (31/148)</td>
<td>22.3 (27/121)</td>
<td>24.4 (42/172)</td>
<td>24.6 (17/69)</td>
</tr>
</tbody>
</table>

NOTE. Data are percentage (proportion) of subjects, unless otherwise noted. AL, artemether-lumefantrine; AS, artesunate; AQ, amodiaquine; GM, geometric mean; IQR, interquartile range; SP, sulfadoxine-pyrimethamine.

Table 2. Outcomes for the different regimens on day 28 after initiation of treatment.

<table>
<thead>
<tr>
<th>Treatment outcome</th>
<th>SP monotherapy (n = 143)</th>
<th>SP plus AQ (n = 118)</th>
<th>SP plus AS (n = 160)</th>
<th>AL (n = 75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adequate clinical response</td>
<td>44.1</td>
<td>87.0</td>
<td>81.9</td>
<td>96.0</td>
</tr>
<tr>
<td>Early treatment failure</td>
<td>9.8</td>
<td>0.9</td>
<td>0.6</td>
<td>0.0</td>
</tr>
<tr>
<td>Late treatment failure</td>
<td>44.8</td>
<td>7.8</td>
<td>13.8</td>
<td>2.0</td>
</tr>
<tr>
<td>reinfection</td>
<td>1.4</td>
<td>4.3</td>
<td>3.8</td>
<td>2.0</td>
</tr>
</tbody>
</table>

NOTE. Data are percentage of subjects with the indicated outcome; n indicates the no. of subjects evaluated. AL, artemether-lumefantrine; AS, artesunate; AQ, amodiaquine; SP, sulfadoxine-pyrimethamine.
Contents Marked as Excerpted

During the entire study period, gametocyte prevalence by Pfs25 QT-NASBA was lower in the SP plus AQ, SP plus AS, and AL arms than in the SP monotherapy arm. The children treated with AL had the lowest gametocyte prevalence by Pfs25 QT-NASBA, although the difference between the prevalence in this arm and that in the SP plus AS arm was not statistically significant (figure 2B).

**Infectiousness of children in different treatment arms.** A total of 118 successful membrane-feeding assays were conducted for children who had microscopically confirmed gametocytemia on day 7 and were >2 years of age. Three assays were not included in the analyses because <20 mosquitoes were dissected. A high percentage of the gametocyte carriers were infectious to mosquitoes (table 3). The estimated percentage of the population who were infectious to mosquitoes was significantly lower after treatment with either SP plus AS or AL, compared with that after SP monotherapy. No statistically significant difference was observed between SP monotherapy and treatment with SP plus AQ. However, the high gametocyte prevalence by Pfs25 QT-NASBA prompted us to randomly test blood samples from treated children by the membrane-feeding assay. Across treatment arms, the percentages of the randomly selected children who were infectious to mosquitoes were consistently higher than the estimated percentages of the children with microscopically confirmed gametocytemia (table 3). Although this result was observed for SP monotherapy (64.0% vs. 48.4%), the relative increase was most evident for the combination therapies, for which the infectious proportion increased 2-fold for SP plus AQ treatment (80.0% vs. 37.9%), 3-fold for SP plus AS treatment (44.0% vs. 14.7%), and 4-fold for AL treatment (60.0% vs. 14.0%). Among the randomly selected children themselves, there were no statistically significant reductions in the infectious proportion for treatment with SP plus AQ, SP plus AS, or AL, compared with that for SP monotherapy (table 3).

The force of malaria infection in the natural situation is determined by the infectious mosquito reservoir. This reservoir is related to the infectious proportion of the human population but also to the percentage of mosquitoes that become infected when feeding on humans. Among the randomly selected children, the percentage of mosquitoes that became infected was

low-up, gametocyte prevalence by microscopy was significantly lower among the children in the SP plus AQ, SP plus AS, and AL arms than among the children in the SP monotherapy arm (figure 2A). Gametocyte prevalence by Pfs25 QT-NASBA was much higher than by microscopy (figure 2B). The overall gametocyte prevalence was 22.9% (117/510) at enrollment by microscopy, compared with 89.4% (219/245) by Pfs25 QT-NASBA ($\chi^2 = 295.9; P < .001$). Although gametocyte prevalence by Pfs25 QT-NASBA was almost universal at enrollment (range for the treatment arms, 85%–92%), it decreased gradually during the follow-up period for the SP plus AQ ($\beta = -0.060 \pm 0.014; P < .001$), SP plus AS ($\beta = -0.090 \pm 0.012; P < .001$), and AL ($\beta = -0.099 \pm 0.015; P < .001$) arms but not for the SP monotherapy arm ($\beta = -0.0032 \pm 0.015; P = .83$).

**Figure 2.** Gametocyte prevalence by different methods of detection. A. Gametocyte prevalence by microscopy after sulfadoxine-pyrimethamine (SP) monotherapy (black diamonds), SP plus amodiaquine (AQ) treatment (white triangles), SP plus artesunate (AS) treatment (white squares), and arteether-lumefantrine (AL) treatment (black triangles). Bars indicate 95% confidence intervals (CIs). Differences in gametocyte prevalence were statistically significant for the comparisons between SP monotherapy and SP plus AS ($\beta = -0.78 \pm 0.17; P < .001$), SP monotherapy and AL ($\beta = -1.14 \pm 0.26; P < .001$), SP plus AQ and SP plus AS ($\beta = -0.70 \pm 0.19; P < .001$), and SP plus AQ and AL ($\beta = -1.06 \pm 0.27; P < .01$). Differences in gametocyte prevalence were not statistically significant for the comparisons between SP monotherapy and SP plus AQ ($\beta = -0.08 \pm 0.18; P = .67$) and between SP plus AS and AL ($\beta = -0.35 \pm 0.27; P = .18$). B. Gametocyte prevalence by Pfs25 RNA–based quantitative nucleic acid sequence–based amplification after SP monotherapy (black diamonds), SP plus AQ treatment (white triangles), SP plus AS treatment (white squares), and AL treatment (black triangles). Bars indicate 95% CIs. Differences in gametocyte prevalence were statistically significant for the comparisons between SP monotherapy and SP plus AS ($\beta = -1.31 \pm 0.38; P = .001$), SP monotherapy and SP plus AS ($\beta = -2.14 \pm 0.33; P < .001$), SP monotherapy and AL ($\beta = -2.44 \pm 0.37; P < .001$), SP plus AQ and SP plus AS ($\beta = -0.83 \pm 0.30; P = .007$), and SP plus AQ and AL ($\beta = -1.12 \pm 0.34; P = .001$). The difference in gametocyte prevalence between SP plus AS and AL was not statistically significant ($\beta = -0.30 \pm 0.27; P = .27$).
Table 3. Percentages of infectious children, as determined by membrane-feeding assays, in microscopically confirmed gametocyte carriers and in a random selection of subjects, by treatment arm.

<table>
<thead>
<tr>
<th>Group, treatment arm</th>
<th>Children with gametocytemia on day 7</th>
<th>Infectious gametocyte carriers</th>
<th>Infectious childrena</th>
<th>RR (95% CI)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopically confirmed gametocyte carriers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP monotherapy</td>
<td>61.5 (83/135)</td>
<td>78.7 (48/61)</td>
<td>48.4</td>
<td>1</td>
</tr>
<tr>
<td>SP plus AQ</td>
<td>46.2 (55/119)</td>
<td>82.1 (23/28)</td>
<td>37.9</td>
<td>0.79 (0.59–1.05)</td>
</tr>
<tr>
<td>SP plus AS</td>
<td>23.8 (39/164)</td>
<td>61.9 (13/21)</td>
<td>14.7</td>
<td>0.30 (0.20–0.46)</td>
</tr>
<tr>
<td>AL</td>
<td>16.0 (12/75)</td>
<td>87.5 (7/8)</td>
<td>14.0</td>
<td>0.30 (0.17–0.53)</td>
</tr>
<tr>
<td>Randomly selected children</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP monotherapy</td>
<td>NA</td>
<td>NA</td>
<td>64.0 (16/25)</td>
<td>1</td>
</tr>
<tr>
<td>SP plus AQ</td>
<td>NA</td>
<td>NA</td>
<td>80.0 (20/25)</td>
<td>1.25 (0.88–1.78)</td>
</tr>
<tr>
<td>SP plus AS</td>
<td>NA</td>
<td>NA</td>
<td>44.0 (11/25)</td>
<td>0.69 (0.40–1.17)</td>
</tr>
<tr>
<td>AL</td>
<td>NA</td>
<td>NA</td>
<td>60.0 (15/25)</td>
<td>0.94 (0.61–1.45)</td>
</tr>
</tbody>
</table>

NOTE. Data are percentage (proportion) of subjects, unless otherwise noted. The median no. of dissected mosquitoes per assay was 31 (interquartile range, 30–48) for the microscopically confirmed gametocyte carriers and was not different between treatment arms (Kruskal-Wallis \( \chi^2 = 0.88; P = .83 \)). The no. of dissected mosquitoes for the randomly selected children was exactly 30 for each assay. AL, artemether-lumefantrine; AQ, amodiaquine; AS, artesunate; CI, confidence interval; NA, not applicable; RR, relative risk; SP, sulfadoxine-pyrimethamine.

a The percentage of infectious children was estimated for the microscopically confirmed gametocyte carriers by multiplying the percentage of children with gametocytemia on day 7 after initiation of treatment and the percentage of infectious gametocyte carriers. The no. of children who were included in the membrane-feeding assays was less than the no. of children with gametocytemia because only children \( \geq 2 \) years of age were eligible for the membrane-feeding assays. For the randomly selected children, the infectious proportion was determined directly.

b The RR for the probability of a child being infectious, with the SP monotherapy arm as the reference group.

quantified for each treatment arm and was found to be 6.9% for SP monotherapy, 5.5% for SP plus AQ treatment, 2.3% for SP plus AS treatment, and 3.6% for AL treatment (table 4). Compared with that for SP monotherapy, the probability of a mosquito becoming infected was significantly lower for treatment with SP plus AS and with AL but not for treatment with SP plus AQ.

**DISCUSSION**

The present study shows that the effect of ACT on the infectiousness of young children to mosquitoes is moderate in an area of Kenya where malaria is highly endemic. Although ACT reduces the density of gametocytemia and the proportion of infected mosquitoes, submicroscopic gametocytemia appears to be sufficient to drive posttreatment malaria transmission.

Our microscopy findings on posttreatment gametocyte prevalence are similar to those of other studies of SP monotherapy [7, 23] and of treatment with SP plus AS [7, 23, 27, 28], SP plus AQ [6, 23, 27], and AL [6, 10]. The infectiousness of gametocyte carriers was somewhat higher in our study than in the studies by Targett et al. [7] and Sutherland et al. [10], possibly because the membrane-feeding assays were conducted on different days. We conducted ours on day 14 after initiation of treatment, whereas Targett et al. and Sutherland et al. conducted theirs on day 7, when gametocytes may not be fully mature [22] or may be less infectious as a result of residual drug concentrations [29]. We nevertheless consider this possibility to be unimportant for interpretation of our data, because our findings on infectiousness by microscopy are comparable with those of these 2 previous studies and lead to the same conclusions. We observed a clear reduction in malaria transmission after administration of ACT (i.e., treatment with SP plus AS [7] or with AL [10]). No estimate for infectiousness has been given previously for SP plus AQ treatment, which appears to be similar to SP monotherapy with respect to its effect on posttreatment malaria transmission.

The results of the membrane-feeding assays for the randomly selected children, however, lead to very different conclusions. The most likely explanation for this is that detection of gametocytes by microscopy represents only the tip of the iceberg. The results for the randomly selected children suggest that relying on microscopy to select gametocyte carriers may lead to an overestimation of the effects of treatment regimens on malaria transmission. This was evident for all tested regimens and is attributable to low-density gametocytemia. The proportion of gametocyte carriers among children presenting with clinical malaria was as much as 4-fold higher when based on PfS25 QT-NASBA than when based on microscopy, and certainly this estimate is closer to the truth. This proportion decreased markedly after administration of ACT, suggesting a direct negative
effect of artemisinin derivatives on gametocyte survival [10, 30]. In contrast to SP monotherapy, ACT seemed to limit the period of infectiousness. Despite the evident reduction in gametocytemia after the administration of ACT, during the first 2 weeks after treatment, the majority of children harbored gametocytes at a density that was at or below the microscopic limit of detection, and a large proportion of these carriers were capable of infecting mosquitoes. The observation of infectiousness in children with gametocytemia below the microscopic threshold is not unexpected [31–33], but the relative contribution made by these carriers to posttreatment malaria transmission in the present study is remarkable. Although a recent study concluded that AL treatment is highly effective in preventing posttreatment malaria transmission [10], we found here that 60% of children treated with AL were capable of infecting mosquitoes. The present study shows that ACT does not substantially reduce the proportion of infectious children but results in a significantly lower proportion of infected mosquitoes. In this manner, ACT is likely to have beneficial effects on transmission.

Submicroscopic gametocytemia is a relevant factor in mathematical models of general malaria transmission [34] and the development and spread of drug resistance [35]. Resistance of malarial parasites to SP is common in our study area, with more than half of the children experiencing treatment failure during follow-up. This finding is similar to those of recent studies in Kenya and Uganda [27, 36, 37]. The efficacy of SP combination therapy is high, as has been previously reported for SP plus AQ treatment [27, 38] and SP plus AS treatment [27]. Here, we found that AL treatment provides the best clinical response, with only 2% treatment failure; this is identical to recent findings from Uganda and Tanzania [6, 39].

Transmission intensity varies significantly in Africa and is determined by the local reservoir of infectious mosquitoes and the gametocyte prevalence in a population. We dissected ~50 fully fed mosquitoes per child, which is approximately equivalent to 1 week of exposure to mosquito bites in our study area [40]. Considering a person’s infectious period, this makes our estimates reasonable for the local situation, but our results clearly need to be interpreted in the context of biting rates in areas with other levels of endemicity. In our study area, the gametocyte prevalence at enrollment was ~90%. In a previous study of malaria transmission after AL treatment in the Gambia [10], where transmission intensity is much lower and highly seasonal, a number of children without microscopically detectable gametocytemia before or after treatment were included in membrane-feeding assays. Only 3.2% (1/31) of these assays resulted in mosquito infection (G. Targett, R. Ord, M. Jawara, and C. Sutherland, unpublished data), which is much lower than our present findings, despite comparable levels of infectiousness among children with microscopically confirmed gametocytemia. This suggests that infectious submicroscopic gametocyte densities may be less common in the Gambia. Findings from an area of low endemicity in Sudan, on the contrary, suggested a high prevalence (12%–45%) of submicroscopic gametocyte densities, although infectiousness was not assessed [41]. Future studies conducted in the general populations of areas with different transmission intensities and seasonalities should determine the relationships among the level of endemicity, the prevalence of submicroscopic gametocytemia, and infectiousness to mosquitoes.

Although Pfs25 QT-NASBA is much more sensitive than microscopy and may prove to be very valuable in estimating gametocyte prevalences, some reservations should be considered. It is not the mere presence of gametocytes but their capacity to infect mosquitoes that is relevant for transmission. For this, the accepted assay is measurement by membrane feeding. In addition, the sensitivity of Pfs25 QT-NASBA is 20–100 gametocytes/mL (i.e., 0.02–0.1 gametocytes/μL). The average size of a blood meal of a mosquito is 3 μL, and, to result in mosquito infection, it should contain a minimum of 1 male and 1 female gametocyte. Therefore, the lowest densities detected by Pfs25 QT-NASBA may be too low to be relevant for malaria transmission.

In conclusion, our findings need to be interpreted in the context of transmission intensity but indicate that none of the tested drug regimens can clear gametocytes from all children during the month after treatment. ACTs in general, and AL in particular, are efficacious as treatments for uncomplicated malaria and able to limit the period of posttreatment gametocyte carriage. However, because of the contribution made by submicroscopic gametocytemias, infectiousness appears to be the rule rather than the exception after treatment. These findings highlight the possible limitations of interventions that aim to reduce transmission by use of antimalarial drugs and indicate

### Table 4. Percentage of mosquitoes that became infected in membrane-feeding assays, by treatment arm.

<table>
<thead>
<tr>
<th>Treatment arm</th>
<th>Infected mosquitoes, % (proportion)</th>
<th>RR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP monotherapy</td>
<td>6.9 (52/750)</td>
<td>1</td>
</tr>
<tr>
<td>SP plus AQ</td>
<td>5.5 (41/750)</td>
<td>0.79 (0.53–1.17)</td>
</tr>
<tr>
<td>SP plus AS</td>
<td>2.3 (17/750)</td>
<td>0.33 (0.19–0.56)</td>
</tr>
<tr>
<td>AL</td>
<td>3.6 (27/750)</td>
<td>0.52 (0.33–0.82)</td>
</tr>
</tbody>
</table>

*The RR for the probability of a mosquito becoming infected, with the SP monotherapy arm as the reference group.
that gametocyte detection by microscopy is insufficient for future studies of malaria transmission.

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