Malaria Transmission After Artemether-Lumefantrine and Dihydroartemisinin-Piperaquine: A Randomized Trial


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(See the editorial commentary by Price on pages 1627–9.)

Background. Artemisinin-based combination therapy (ACT) reduces the potential for malaria transmission, compared with non-ACTs. It is unclear whether this effect differs between ACTs.

Methods. A total of 298 children (age, 6 months to 10 years) with uncomplicated falciparum malaria were randomized to artemether-lumefantrine (AL; n = 153) or dihydroartemisinin-piperaquine (DP; n = 145) in Mbita, a community in western Kenya. Gametocyte carriage was determined by molecular methods on days 0, 1, 2, 3, 7, 14, 28, and 42 after treatment initiation. The gametocyte infectiousness to mosquitoes was determined by mosquito-feeding assays on day 7 after beginning therapy.

Results. The cumulative risk of recurrent parasitemia on day 42 after initiation of treatment, unadjusted by polymerase chain reaction findings, was 20.7% (95% confidence interval [CI], 14.4–28.2) for AL, compared with 3.7% (95% CI, 1.2–8.5) for DP (P < .001). The mean duration of gametocyte carriage was determined by molecular methods on days 0, 1, 2, 3, 7, 14, 28, and 42 after treatment initiation. The gametocyte infectiousness to mosquitoes was determined by mosquito-feeding assays on day 7 after beginning therapy.

Conclusions. While DP was associated with a longer prophylactic time after treatment, gametocyte carriage and malaria transmission to mosquitoes was lower after AL treatment.

Clinical Trials Registration: NCT00868465.

Keywords. malaria; falciparum; artemisinin; coartem; transmission; anopheles; mosquito; recrudescence; genotyping; gametocyte.

Artemisinin-based combination therapy (ACT) has been adopted as first-line treatment for uncomplicated malaria throughout Africa. Despite concerns about increasing resistance to artemisinins in Thailand and Cambodia [1, 2], ACTs continue to have excellent cure rates in Africa [3]. An important benefit of ACTs is their effect on gametocytes, which, when ingested by mosquitoes, trigger sexual reproduction of parasites. Gametocyte carriage and posttreatment malaria transmission is reduced after receipt of ACTs [4–6]. These transmission-reducing properties have been associated with sharp reductions in malaria transmission intensity and malaria incidence in several African settings following the
introduction of ACTs [7–10]. While there is indisputable evidence that, compared with non-ACTs, ACT reduces the potential for malaria transmission [11], it is currently unclear whether this transmission-reducing effect differs among ACTs. Artemether-lumefantrine (AL) is the most widely used ACT in Africa. Dihydroartemisinin-piperaquine (DP) may be equally efficacious [3, 12] and has advantages of simpler dosing and a longer prophylactic period [3, 12].

There is conflicting evidence about the comparative effect of AL and DP on posttreatment gametocyte carriage. Some studies indicate a longer duration of microscopically detected gametocyte carriage after AL treatment [12–14], whereas others indicate a similar [15] or shorter duration [3, 16, 17]. A large multicenter ACT trial indicated that definitive conclusions about the malaria transmission potential after different ACTs are currently unavailable and cannot be based on microscopy alone [3] since microscopy only detects a fraction of all gametocytes [17]. Individuals with gametocyte densities below the microscopic threshold for detection have repeatedly been shown to be infectious to mosquitoes [18–20], and a minimum gametocyte density that is required for successful mosquito infection has not been established. Importantly, microscopy and other currently available gametocyte detection tools do not allow inferences on the infectiousness of gametocytes to mosquitoes to be made. The infectiousness of gametocytes that are observed after initiation of treatment may vary between treatment regimens: most antimalarials clear immature gametocytes but leave highly infectious mature gametocytes unaffected [21], whereas others appear to induce the production or release of less infectious gametocytes [6, 22, 23]. Mosquito-feeding assays are the only currently available tools to provide direct evidence of the infectiousness of gametocytes.

We used a highly sensitive molecular assay to determine the duration of gametocyte carriage among children who received AL or DP, and, to our knowledge, we are the first to have directly determined gametocyte transmission to mosquitoes that fed on posttreatment blood samples.

METHODS

Study Design and Participants
This study was conducted in Mbita, a community in western Kenya, from April to June 2009. The study area is characterized by moderate transmission intensity [4]. Previous trials with AL and DP in 2004 and 2007 found an adequate clinical response over 28 days in 82%–100% of treated children, with all patients exhibiting microscopy-confirmed clearance of parasitemia by day 2 after initiation of treatment [4, 14]. Children from 6 months to 10 years old were included when they had either a tympanic temperature of ≥37.5°C or a history of fever in the last 24 hours and microscopically confirmed Plasmodium falciparum infection with an asexual parasite density of 1000–200 000 parasites/μL. Exclusion criteria were a hemoglobin level of <5 g/dL, the presence of other disease that causes febrile conditions, the presence of any other Plasmodium species, a history of adverse events against either of the study drugs, or signs of severe malaria. The protocol received ethical approval from the Ethical Review Committee of the Kenya Medical Research Institute and the ethics committee of the London School of Hygiene and Tropical Medicine. Written informed consent was obtained from a parent or guardian of the participating children.

Interventions and Randomization
Children were weighed and randomly allocated to receive (1) 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 hours [±90 minutes] after initiation of treatment) or (2) DP (Duocotexin, Holley Pharm, 40 mg dihydroartemisinin/320 mg piperaquine tablets) administered as a targeted total dose of 6.4 and 51.2 mg/kg of dihydroartemisinin and piperaquine, respectively, given in 3 equally divided daily doses to the nearest half tablet. The quality of the drugs used in this trial was confirmed by the methods described by Kaur et al [24]. All treatment doses were given under direct supervision with local fatty food to facilitate absorption. A randomization list was generated for different age strata (<2 years, 2–5 years, and 5–10 years), using MS Excel, targeting allocation to each of the study arms at a ratio of 1:1. Except for those involved in administering medication, all staff members engaged in the trial were blinded to the treatment arm to which each child was assigned.

Procedures
Children were examined at the study clinic on days 1, 2, 3, 7, 14, 28, and 42 after enrollment and on any other day that the child became ill. If children were parasitemic at any time point after day 3, they received rescue treatment with mefloquine and were excluded from further follow-up. A single finger-stick specimen was collected on all follow-up days after enrollment and was used for preparation of a microscopic slide (on all days except day 1), a 50-μL microtainer blood sample, and a filter paper blood spot (903 and 3MM Whatman, Maidstone, UK). All blood smears were Giemsa stained, and 100 microscopic fields were read by 2 microscopists for asexual parasites, with a third microscopist used if results differed by >25%. At enrollment and on day 7, the day of membrane feeding, slides were re-read for gametocytes by 2 independent microscopists. Gametocyte detection was done for a random selection of individuals on all days of follow-up by quantitative nucleic acid sequence-based amplification (QT-NASBA) as described elsewhere, with an approximate detection limit of 0.1 gametocytes/μL [25]. MSP-1 and MSP-2 genotyping was performed using primers described by Snounou et al [26] after DNA extraction by the Chelex method [27] or the QiaAmp DNA Micro kit (Qiagen, UK) to
distinguish between recrudescence and reinfections on paired filter paper samples from enrollment and the follow-up day on which parasites were detected by microscopy [28]. These 2 markers were previously shown to be discriminative in the study region [4, 29].

On day 7 after initiation of treatment, all children aged ≥2 years were invited for membrane-feeding assays. Additional written informed consent was obtained from a parent or guardian of children participating in membrane-feeding assays. Children were enrolled in membrane-feeding assays in the order in which they appeared at the clinic; the maximum number of experiments that was conducted per day depended on mosquito husbandry. A 3-mL venous blood sample was obtained; if venipuncture failed twice, the child was not enrolled in the membrane-feeding experiment. Blood samples were fed to approximately 100 locally reared 4–5-day-old female Anopheles gambiae sensu stricto mosquitoes via an artificial membrane-feeder system [4]. One week later, up to 30 surviving mosquitoes per experiment were examined for oocysts by 2 independent microscopists. A third microscopist was consulted if the 2 microscopists disagreed, and the majority result was recorded.

Sample Size Calculations
We calculated a sample size that was sufficient to test the hypothesis that the risk of recurrent parasitemia after 42 days would differ between AL-treated children and DP-treated children. A total of 150 patients (allowing for 10% loss to follow-up) needed to be enrolled in each treatment arm to detect a difference of 20% in the risk of recurrent parasitemia, assuming a 42-day risk of recurrent parasitemia (unadjusted by genotyping) of 50% after AL treatment [30], with a 2-sided type I error of 0.05 and a power of 90%. A random selection was made for QT-NASBA analysis, using computer-randomized tables. The number of individuals included in QT-NASBA analysis was based on an estimated duration of gametocyte carriage (±SD) of 13.4 ± 7.5 days [31] after AL treatment, with a 65% longer duration of carriage after DP treatment [3]. Inclusion of 47 children per treatment arm, each contributing 1 estimate of the duration of gametocyte carriage, would allow us to detect this difference with a 2-sided type I error of 0.05 and a power of 95%. The number of children included in membrane-feeding assays was not based on sample size calculations but on the maximum that was logistically feasible on the basis of mosquito husbandry. Previous studies have included 10–61 experiments per treatment arm [4, 5].

Data Analysis
The primary outcome was the parasitological efficacy of AL and DP. The time to treatment failure, defined as the time to the appearance of asexual parasites during follow-up, without adjustment for polymerase chain reaction (PCR) findings, was compared between treatment arms by Cox proportional hazard models. The number of PCR-confirmed recrudescence was compared between treatment arms by the Fisher exact test and logistic regression models; for this purpose, only infections that were classified as recrudescence after PCR adjustment by MSP-1 and MSP-2 genotyping findings were considered treatment failures.

Secondary outcomes were (submicroscopic) gametocyte carriage and malaria transmission to mosquitoes. The mean duration of gametocyte carriage after treatment for individuals with an adequate clinical response was estimated using a previously published mathematical model for repeated QT-NASBA measurements [31]. The main advantage of this model is that it allows for the release of gametocytes from sequestration. The disappearance of gametocytes during follow-up for individuals who carried gametocytes at enrollment, as detected by QT-NASBA, was determined using a Kaplan-Meier estimator; the log-rank test was used to compare curves for AL and DP. The proportion of gametocytemic individuals and the proportion of infectious individuals (ie, subjects who infected at least 1 mosquito) were compared between treatment arms by the χ² test and logistic regression models. The proportion of infected mosquitoes, the oocyst burden in mosquitoes, and the number of gametocyte-positive days were compared between groups by negative binomial or logistic regression models, using generalized estimating equations to adjust for clustering between observations from the same individual.

Because the dose of DP received was previously associated with the risk of recrudescence or reinfection [32, 33], we calculated the total actual doses of lumefantrine and piperaquine received over the 3 treatment days and related this to treatment outcome.

RESULTS
Of the 2073 screened children, 298 met the enrollment criteria and were randomly assigned to receive treatment with AL or DP (Figure 1). Geometric mean asexual parasite density at enrollment was 15,360 parasites/μL (95% confidence interval [CI], 13,432–17,564 parasites/μL) and did not differ between treatment arms (P = .28; Table 1). Enrollment gametocyte prevalence was 9.7% (26 of 267) by microscopy and 71.3% (67 of 94) by QT-NASBA and did not differ between treatment arms (P ≥ .49; Table 1). The mean total dose of lumefantrine in the AL arm (±SD) was 65.5 ± 8.4 mg/kg body weight (range, 51.4–102.9 mg/kg body weight); the mean total dose of piperaquine in the DP arm (±SD) was 60.2 ± 10.7 mg/kg body weight (range, 48.0–87.3 mg/kg body weight).

Primary Analysis: Efficacy of AL and DP
By day 42 after initiation of treatment, 20.7% of the children (30 of 145) in the AL arm were parasite positive by microscopy, compared with 3.7% (5 of 134) in the DP arm (hazard ratio, 0.17; 95% CI, .07–.44; P < .001; Table 2). This failure rate was
not statistically significantly associated with enrollment parasite density \((P = .38)\), age \((P = .94)\), total dose of lumefantrine \((P = .31)\), or total dose of piperaquine \((P = .64)\). MSP-1 and MSP-2 genotyping was successful for 30 of 35 recurrent infections; 3 samples failed to amplify on the day of failure or the preceding visit, and 2 infections had missing samples. Only 4 of these 30 samples, all from the AL arm \((P = .053)\), were classified as recrudescence, and the remainder were classified as new infections.

By microscopy, all children cleared their asexual parasites by day 7 after treatment. On day 2 after treatment, before the fifth dose of AL or the third dose of DP was administered, 3.3% of children (5 of 150) in the AL arm and 7.3% (10 of 138) in the DP arm had residual asexual parasitemia detected by microscopy \((P = .14)\). On day 3 after initiation of treatment, 1 child in the DP arm had residual asexual parasitemia of 150 parasites/\(\mu\)L detected by microscopy, down from an initial asexual parasite density of 3500 parasites/\(\mu\)L.

### Secondary Analysis: Posttreatment Malaria Transmission Potential

QT-NASBA–determined gametocyte prevalence declined during follow-up (Figure 2). The gametocyte prevalence increased on days 28 and 42 of follow-up and was strongly associated with the concurrent presence of asexual parasites on these days. On day 42 after initiation of treatment, the QT-NASBA–determined gametocyte prevalence was 45.5% (5 of 11) for children who had asexual parasites detected by microscopy on that day, compared with 5.4% (2 of 37) for parasite-free children \((P = .001)\). The number of gametocyte-positive days was lower for AL-treated children (32.0% [110 of 344]) than for DP-treated children (42.9% [127 of 296]; \(P = .008)\). The mean duration of gametocyte carriage for children who remained free of asexual parasites during follow-up was 5.5 days (95% CI, 3.6–8.5) for the AL group and 15.3 days (95% CI, 9.7–24.2) for the DP group \((P = .001)\). Some individuals harbored gametocytes considerably longer (Figure 3). When analyses were restricted to individuals...
who were gametocyte positive by QT-NASBA at enrollment, the time to disappearance of gametocytes was significantly shorter for the AL group, compared with the DP group (hazard ratio, 2.35; 95% CI, 1.19–4.66; *P* = .01; Figure 3).

One hundred and sixty-two children agreed to donate blood for membrane-feeding experiments. Venipuncture failed for 5 children; for the other 157 experiments, a minimum of 17 mosquitoes were examined on day 7 after feeding (median, 30

### Table 1. Characteristics of the Study Participants at Enrollment, by Study Arm

<table>
<thead>
<tr>
<th>Variable</th>
<th>AL</th>
<th>DP</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Children, no.</td>
<td>153</td>
<td>145</td>
<td></td>
</tr>
<tr>
<td>Male sex</td>
<td>51.0 (78/153)</td>
<td>51.0 (74/145)</td>
<td></td>
</tr>
<tr>
<td>Age, y, median (IQR)</td>
<td>5 (3–8)</td>
<td>5 (3–7)</td>
<td></td>
</tr>
<tr>
<td>Temperature ≥37.5°C</td>
<td>43.1 (66/153)</td>
<td>41.4 (60/145)</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin level, mmol/dL, mean (95% CI)</td>
<td>6.58 (6.39–6.78)</td>
<td>6.40 (6.20–6.61)</td>
<td></td>
</tr>
<tr>
<td>Asexual parasite density, parasites/μL, geometric mean (95% CI)</td>
<td>15 840 (13 213–18 990)</td>
<td>15 160 (12 415–18 512)</td>
<td></td>
</tr>
<tr>
<td>Microscopy finding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gametocyte prevalence</td>
<td>8.8 (12/137)</td>
<td>10.8 (14/130)</td>
<td></td>
</tr>
<tr>
<td>Gametocyte density, a gametocytes/μL, geometric mean (95% CI)</td>
<td>64.0 (33.7–121.5)</td>
<td>64.7 (37.2–112.4)</td>
<td></td>
</tr>
<tr>
<td>Pfs25 QT-NASBA finding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gametocyte prevalence</td>
<td>68.1 (32/47)</td>
<td>74.5 (35/47)</td>
<td></td>
</tr>
</tbody>
</table>

Data are % (proportion) of participants, unless otherwise indicated.

Abbreviations: AL, artemether-lumefantrine; CI, confidence interval; DP, dihydroartemisinin-piperaquine; IQR, interquartile range; QT-NASBA, quantitative nucleic acid sequence–based amplification.

a Data are for gametocyte carriers only.

### Table 2. Treatment Outcomes on Days 28 and 42 After Initiation of Treatment, by Study Arm

<table>
<thead>
<tr>
<th>Outcome, by Day</th>
<th>AL</th>
<th>DP</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 28 Observations, no.</td>
<td>147</td>
<td>137</td>
<td></td>
</tr>
<tr>
<td>Treatment outcome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adequate clinical response</td>
<td>93.2 (137/147)</td>
<td>100.0 (0/137)</td>
<td>.002</td>
</tr>
<tr>
<td>Early treatment failure</td>
<td>0.0 (0/147)</td>
<td>0.0 (0/147)</td>
<td></td>
</tr>
<tr>
<td>Late treatment failure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overallb</td>
<td>6.8 (10/147)</td>
<td>0.0 (0/137)</td>
<td></td>
</tr>
<tr>
<td>Due to recrudescenceb</td>
<td>1.4 (2/147)</td>
<td>0.0 (0/137)</td>
<td></td>
</tr>
<tr>
<td>Due to new infectionb</td>
<td>5.4 (8/147)</td>
<td>0.0 (0/137)</td>
<td></td>
</tr>
<tr>
<td>Indeterminate cause</td>
<td>0.0 (0/147)</td>
<td>0.0 (0/137)</td>
<td></td>
</tr>
<tr>
<td>Day 42 Observations, no.</td>
<td>145</td>
<td>134</td>
<td></td>
</tr>
<tr>
<td>Treatment outcome day 42, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adequate clinical response</td>
<td>79.3 (115/145)</td>
<td>96.3 (129/134)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Early treatment failure</td>
<td>0.0 (0/145)</td>
<td>0.0 (0/134)</td>
<td></td>
</tr>
<tr>
<td>Late treatment failure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overallb</td>
<td>20.7 (30/145)</td>
<td>3.7 (5/134)</td>
<td></td>
</tr>
<tr>
<td>Due to recrudescenceb</td>
<td>2.8 (4/145)</td>
<td>0.0 (0/134)</td>
<td></td>
</tr>
<tr>
<td>Due to new infectionb</td>
<td>15.9 (23/145)</td>
<td>2.2 (3/134)</td>
<td></td>
</tr>
<tr>
<td>Indeterminate cause</td>
<td>2.1 (3/145)</td>
<td>1.5 (2/134)</td>
<td></td>
</tr>
</tbody>
</table>

Data are % (proportion) of observations, unless otherwise indicated.

Abbreviations: AL, artemether-lumefantrine; DP, dihydroartemisinin-piperaquine; PCR, polymerase chain reaction.

a Unadjusted by PCR findings.

b Adjusted by PCR-based MSP-1 and MSP-2 genotyping findings for samples collected at enrollment and on the day of failure.
mosquitoes; interquartile range, 30–30 mosquitoes). For 151 of these experiments, slide results were available; 80.0% of children (12 of 15) who had gametocytes detected by microscopy in the feed sample infected at least 1 mosquito, compared with 29.4% (40 of 136) with slides that were negative for gametocytes \((P < .001)\). Gametocyte carriage detected by QT-NASBA was associated with the likelihood of infecting at least 1 mosquito. Of children who were gametocyte positive by QT-NASBA, 66.7% (18 of 26) infected at least 1 mosquito, compared with 23.7% of children (9 of 38) who were gametocyte negative by QT-NASBA \((P < .001)\). Infectiousness to mosquitoes was not significantly associated with fever at enrollment \((P = .44)\) or fever on the day of membrane feeding \((P = .73)\).

The proportion of individuals that infected at least 1 mosquito was not significantly different between treatment arms \((P = .40; \text{Table } 3)\). However, the proportion of infected mosquitoes and oocyst intensity were different between treatment arms. In the AL arm, 1.9% of mosquitoes (44 of 2293) became infected with 1–2 oocysts per mosquito midgut. In the DP arm, 3.5% of mosquitoes (84 of 2371) became infected with 1–14 oocysts. Treatment with DP was associated with a borderline significantly higher proportion of infected mosquitoes \((\text{odds ratio, } 1.96; 95\% \text{ CI, } .96–3.97; P = .06)\) and a significantly higher oocyst burden in infected mosquitoes \((\text{incidence rate ratio, } 2.71; 95\% \text{ CI, } 1.34–5.47; P = .005)\), after adjustment for correlation between observations from the same individual.

**DISCUSSION**

In this study, we present the first direct comparison of the malaria transmission–reducing effects of 2 leading ACTs. Compared with DP, treatment with AL was associated with a 3-fold shorter duration of gametocyte carriage after initiation of treatment and a significantly lower infectiousness to mosquitoes on day 7 after initiation of treatment.

In line with a recent multicenter study on the efficacy of ACTs in Africa, we found high efficacies of AL and DP [3]. While 35 individuals experienced parasitemia after initial parasite clearance by treatment, MSP-1 and MSP-2 genotyping confirmed only 4 cases of recrudescence, all of which occurred in the AL arm. DP thereby had a significantly higher efficacy than AL. In addition to a better efficacy in preventing recrudescence, our findings also indicated a lower rate of reinfections in the DP arm [3, 12, 13, 15]. This finding is in line with all published literature on the prophylactic period after ACTs and is plausibly a result of the longer elimination half-life of piperaquine (23–28 days), compared with that for lumefantrine (3.2 days) [34, 35]. The longer prophylactic period following treatment is an important advantage for the individual patient in areas of moderate-to-intense malaria transmission where reinfection is likely [36], but it may result in a longer period with subtherapeutic drug levels, during which selection for resistant parasites may occur [37]. In settings of low endemicity, the individual benefit of a longer prophylactic period is relatively small. Drug resistance is more likely to arise in areas of low endemicity [38]; there may therefore be disadvantages of using drugs with a long elimination half-life in these settings. The gametocytocidal properties of ACTs are key in determining their impact on community-wide malaria transmission in settings where malaria

![Figure 2. Gametocyte prevalence by quantitative nucleic acid sequence–based amplification (QT-NASBA) after initiation of treatment with artemether-lumefantrine (AL) or dihydroartemisinin-piperaquine (DP). RNA samples were processed for gametocyte detection by Pfs25 QT-NASBA at enrollment and on days 1, 2, 3, 7, 14, 28, and 42 after initiation of treatment with AL \((n = 47)\) or DP \((n = 47)\). Gametocyte prevalence is given for the different treatment arms; error bars indicate the upper limit of the 95% confidence interval.](https://academic.oup.com/jid/article-abstract/207/11/1637/795056/figure-2)

![Figure 3. Kaplan-Meier plot of the time to disappearance of gametocytes among individuals who were gametocyte positive before treatment with artemether-lumefantrine (AL) or dihydroartemisinin-piperaquine (DP). The time to disappearance of gametocytes was determined for individuals who were gametocyte positive by Pfs25 quantitative nucleic acid sequence–based amplification prior to AL treatment (solid line; \(n = 32\)) or DP treatment (dashed line; \(n = 35\)). \(P = .014\), by the log-rank test.](https://academic.oup.com/jid/article-abstract/207/11/1637/795056/figure-3)
endemicity is low [39]. A long-acting drug with a strong
gametocytocidal activity may have the largest impact across all
levels of transmission intensity. DP has been suggested to fulfill
this role as an ACT with a long-acting partner drug [39].
However, our study shows that the gametocytocidal effect of
DP immediately after treatment was smaller than that of AL.

Gametocytes undergo complex development that is charac-
terized by 5 morphologically distinct stages. The earliest
developmental stages of gametocytes (stage I and II) are suscepti-
tible to most antimalarial drugs, including lumefantrine and
piperaquine [40]. Later-stage gametocytes are unaffected by pi-
péraquine and lumefantrine [40], although a recent study sug-
gested an effect of lumefantrine on mature gametocytes [41].
The active metabolite of artemisinins, dihydroartemisinin, is
highly active against stage I–III gametocytes and has incom-
plete activity against stage IV and V gametocytes [40, 42]. The
artemisinin component is therefore likely to be most important
in determining differences in transmission potential after AL
and DP therapy. A recent multicenter trial comparing 4 dif-
ferent ACTs observed a significantly higher prevalence of micro-
scopically detectable gametocytes after DP therapy but
acknowledged that microscopy only detects a fraction of all ga-
metocytes and does not allow definitive conclusions about
malaria transmission potential [3]. We used a highly sensitive
molecular gametocyte detection tool that, in line with previous
studies, resulted in a 7-fold higher estimation of gametocyte
prevalence at enrollment [17]. We observed that mature game-
tocytes can persist for several weeks after initiation of ACT
treatment [31] and that the duration of gametocyte carriage
was approximately 3-fold shorter for the AL arm, compared
with the DP arm. Repeated assessments of gametocyte carriage
by use of sensitive assays allow incorporation of a longitudinal
element in the estimation of malaria transmission potential but
do not provide conclusive evidence about the infectiousness of
gametocytes. We therefore directly determined posttreatment
infectiousness 1 week after the initiation of treatment in 157 of
298 children, an unsurpassed high proportion of trial partici-
pants. We previously showed that a large proportion of children
are capable of infecting mosquitoes after ACT [4], owing the
longevity of gametocytes [31] and the high efficiency of malaria
transmission at low gametocyte densities [20]. As a conse-
quence, the proportion of individuals capable of infecting at
least 1 mosquito may be similar between treatment arms [4],
whereas the proportion of mosquitoes that become infected
after feeding, which is the transmission outcome with the
largest public health importance, differs as a consequence of
differences in gametocyte density and infectiousness. In this
study, the proportion of mosquitoes that became infected after
feeding on the blood of a treated individual was approximately
2-fold lower after AL treatment, compared with DP treatment.
Ideally, we would have conducted longitudinal assessments of
infectiousness after AL and DP therapy. This would have
allowed us to determine the overall impact of these ACTs with
different gametocytocidal and prophylactic properties on the
malaria transmission potential in our study setting, where
reinfection is frequent. The fact that many children with
asexual parasites during follow-up concurrently harbored ga-
metocytes indicates that rates of drug failure, be it due to recur-
descence or reinfection, have direct implications for transmis-
sibility. In Plasmodium vivax, this association is more
readily appreciated because gametocytes develop early in infec-
tions [17], and a lower efficacy of AL as compared to DP results
in an immediate increased P. vivax transmission potential after
AL [43].

### Table 3. Gametocyte Infectiousness Among Mosquitoes, by Study Arm

<table>
<thead>
<tr>
<th>Variable</th>
<th>AL</th>
<th>DP</th>
<th>P</th>
</tr>
</thead>
</table>
| Individuals participating in membra-
ne-feeding assays, no.                | 77      | 80      |         |
| Microscopy finding on feeding day    |         |         |         |
| Gametocyte prevalence                | 4.2 (3/72)| 15.2 (12/79) | .02 |
| Gametocyte density, gametocytes/μL, geometric mean (95% CI) | 39.5 (18.2–85.4) | 63.8 (38.5–105.8) | .29 |
| Pfs25 QT-NASBA finding on feeding day|         |         |         |
| Gametocyte prevalence                | 21.7 (5/23) | 39.1 (9/23) | .20 |
| Individuals infecting ≥1 mosquito   | 31.1 (24/77) | 36.3 (29/80) | .40 |
| Infected mosquitoes, % (proportion)  | 1.9 (44/2293) | 3.5 (84/2371) | .06b |
| Oocysts in infected mosquitoes, no., mean (range) | 1.3 (1–2) | 1.9 (1–14) | .005bc |

Data are % (proportion) of participants, unless otherwise indicated.

Abbreviations: AL, artemether-lumefantrine; CI, confidence interval; DP, dihydroartemisinin-piperaquine; QT-NASBA, quantitative nucleic acid sequence–based amplification.

aData are for gametocyte carriers only.

bAdjusted for correlations between observations from the same individual.

cDetermined using a negative binomial regression model that incorporated both prevalence and intensity of infection among mosquitoes.
With the currently available data, we can speculate about the plausible impact of AL and DP on *P. falciparum* malaria transmission in different settings. Because of the more pronounced effect of AL on malaria transmission shortly after treatment, our findings suggest that AL may be the most appropriate first-line choice for reducing community-wide transmission of *P. falciparum* in settings of low endemicity. DP may be an appropriate choice to prevent reinfections in areas of higher endemicity.

**Notes**

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