Gametocyte Clearance Kinetics Determined by Quantitative Magnetic Fractionation in Melanesian Children with Uncomplicated Malaria Treated with Artemisinin Combination Therapy

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Quantitative magnetic fractionation and a published mathematical model were used to characterize between-treatment differences in gametocyte density and prevalence in 70 Papua New Guinean children with uncomplicated *Plasmodium falciparum* and/or *Plasmodium vivax* malaria randomized to one of two artemisinin combination therapies (artemether-lumefantrine or artemisinin-naphthoquine) in an intervention trial. There was an initial rise in peripheral *P. falciparum* gametocyte density with both treatments, but it was more pronounced in the artemisinin-naphthoquine group. Model-derived estimates of the median pretreatment sequestered gametocyte population were 21/μl for artemether-lumefantrine and 61/μl for artemisinin-naphthoquine ($P < 0.001$). The median time for *P. falciparum* gametocyte density to fall to $<2.5/\mu\text{l}$ (below which transmission becomes unlikely) was 16 days in the artemether-lumefantrine group and 20 days in artemisinin-naphthoquine group ($P < 0.001$). Gametocyte prevalence modeling suggested that artemisinin-naphthoquine-treated children became gametocytemic faster (median, 2.2 days) than artemether-lumefantrine-treated children (median, 5.3 days; $P < 0.001$) and had a longer median *P. falciparum* gametocyte carriage time per individual (20 versus 13 days; $P < 0.001$). Clearance of *P. vivax* gametocytes was rapid (within 3 days) in both groups; however, consistent with the reappearance of asexual forms in the main trial, nearly 40% of children in the artemether-lumefantrine group developed *P. vivax* gametocytemia between days 28 and 42 compared with 3% of children in the artemisinin-naphthoquine group. These data suggest that artemisinin is less active than artemether against sequestered gametocytes. Greater initial gametocyte release after artemisinin-naphthoquine increases the period of potential *P. falciparum* transmission by 4 days relative to artemether-lumefantrine, but the longer elimination half-life of naphthoquine than of lumefantrine suppresses *P. vivax* recurrence and consequent gametocytemia.

Intraerythrocytic development of *Plasmodium* spp. comprises asexual reproduction, which underlies host pathophysiology or sexual reproduction (gametocyte formation) that is essential for malaria transmission. Apart from distinctive morphology, gametocytes have many characteristics that set them apart from asexual stages. *Plasmodium falciparum* gametocytes have a much longer life span in the circulation and, through metabolic inactivity during the mature phase, reduced antimalarial drug sensitivity (1–3). Artemisinin drugs have greater gametocytocidal activity than conventional agents, such as chloroquine, sulfadoxine, and pyrimethamine, in *P. falciparum* infections (4–7). As their half-lives are relatively short, this greater potency arises largely from their ability to destroy a wider range of early-stage gametocytes, presumably at their sequestration sites (4, 8). However, the exact effect of antimalarial drugs on the viability of more mature *P. falciparum* gametocytes that continue to circulate after treatment is unclear (9, 10).

There are differences in gametocytocidal activity within the artemisinin class. *In vitro* experiments suggest that artemether is less potent than other endoperoxide compounds, including artemisinin itself, in exflagellation assays (11, 12). However, in comparative clinical studies, artemether-based artemisinin combination therapies (ACTs) are at least as effective at reducing post-treatment gametocytemia as artesunate- or dihydroartemisinin

(DHA)-based ACTs (13, 14). With the development of these semi-synthetic derivatives that have more advantageous pharmacological profiles, artemisinin itself has not been incorporated as part of commonly used ACTs, but the first *in vivo* studies showing gametocytocidal activity involved artemisinin (15).

Monitoring posttreatment gametocytemia is an important component of efficacy assessment, and it allows quantification of transmission potential in areas where *Plasmodium* spp. are endemic (1, 16). Sensitive and robust gametocyte detection is crucial

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FIG 1 Schematic representation of the gametocyte models. In the upper density model, sequestered gametocytes (S) are released into circulation after $1/\rho$ days, and peripheral gametocytes (P) are cleared from circulation after $1/\mu$ days. In the lower prevalence model, individuals with sequestered gametocytes (E) become gametocytemic after $1/f$ days, and gametocytemic individuals (I) become gametocyte free after $1/r$ days.

for these purposes (8). The gametocyte density in peripheral blood is often too low for light microscopy (LM) (17), so more sensitive molecular methods have been developed as a result. These include reverse transcriptase PCR (RT-PCR), quantitative nucleic acid sequence-based analysis, and reverse transcriptase loop-mediated amplification (3, 18–23). These methods typically target transcripts of the *Pfs25* gene in *P. falciparum* or its homologue in *Plasmodium vivax* (*Pvs25*). Such molecular methods are not easily transferable to field laboratories, are prone to contamination, use expensive equipment and reagents, and require trained personnel (24, 25). In addition, the development and application of laboratory-derived standard curves relating the transcript copy number to the gametocyte number are difficult given the variability of field isolates (23). As a practical alternative, we have developed and validated a method based on quantitative high-field-gradient magnetic fractionation (QMF) that has similar sensitivity to RT-PCR in laboratory and field settings (24, 26–28).

The aim of the present study was to use QMF to characterize in detail the clearance kinetics of *P. falciparum* and *P. vivax* gametocytes in young children from Papua New Guinea (PNG) who were participating in a randomized trial of two 3-day ACT regimens for uncomplicated malaria, specifically artemether-lumefantrine and artemisinin-naphthoquine (29). Although artemisinin-naphthoquine proved as effective as artemether-lumefantrine therapy (the recommended first-line antimalarial therapy in PNG) for *P. falciparum* malaria, there was increased posttreatment gametocyte carriage in artemisinin-naphthoquine-treated children as assessed by conventional light microscopy (LM) (29). Artemisinin-naphthoquine was superior to artemether-lumefantrine for *P. vivax* malaria, including in the comparison of gametocyte carriage during follow-up. In contrast to a simple analysis of the proportions of patients in the two treatment groups who were gametocyte positive by LM during treatment, we have assessed in the present study the gametocyte sequestration and circulation times, the size of the sequestered gametocyte population, and the gametocyte carriage times using QMF and a previously published mathematical model of gametocyte clearance (4). We hypothesized that the apparent difference in gametocytocidal efficacy between the two ACT regimens reflected differences in clearance of early-stage gametocytes still circulating or present at the sequestration sites, such as bone marrow (30).

MATERIALS AND METHODS

Study site, approvals, and sample collection. The full protocol and initial results of the randomized clinical trial (Australian New Zealand Clinical

Trials Registry ACTRN12610000913077) have been published previously (29). The study received ethical approval from the Medical Research Advisory Committee of the PNG Department of Health (MRAC 10.39). In all cases, informed consent was obtained from the parents or legal guardians of the children before recruitment and blood sampling. The blood samples used in the present study were collected at the Mugil and Alexishafen Health Centers on the north coast of PNG near the township of Madang between March 2011 and April 2013.

The 70 children who participated in the present study were an unselected subset of those in the main trial. They were between ages 0.5 and 5 years and had presented with uncomplicated *P. falciparum* malaria (minimum parasite density, 1,000/ μ l), *P. vivax* malaria (minimum parasite density, 250/ μ l), or a mixed infection with these two *Plasmodium* spp. Randomly assigned treatment comprised either artemether-lumefantrine (1.7:10 mg/kg of body weight twice daily for 3 days) or artemisinin-naphthoquine (20:8 mg/kg daily for 3 days). Venous blood samples were collected on the day of enrollment, and finger-prick mixed capillary blood samples were taken on days 1, 2, 3, 7, 14, 28, and 42 posttreatment.

Determination of gametocyte density. Giemsa-stained thick blood smears for each child and at each time point were examined independently by two skilled microscopists, and the parasitemia was quantified. Discrepancies were adjudicated by a third senior expert microscopist (29). Blood samples for QMF were processed on the day of collection. An aliquot of 200 μ l blood was subjected to QMF, as described elsewhere, using the magnetically activated cell sorting (MACS) system (Miltenyi Biotec, Bergisch Gladbach, Germany) (24, 26). After magnetic fractionation, blood smears were examined by screening the slide for gametocytes, a process which took approximately 5 min using $\times 1,000$ magnification, given that very few cells were present. Gametocytemia was quantified as described previously (28).

Models of gametocyte clearance. The clearance of *P. falciparum* gametocytes was assessed using differential equation systems 1 and 2, as previously described (4) (see Fig. 1).

$$\begin{aligned}\frac{dS}{dt} &= -\rho S \\ \frac{dP}{dt} &= \rho S - \mu P\end{aligned}\quad (1)$$

$$\begin{aligned}\frac{dE}{dt} &= -fE \\ \frac{dI}{dt} &= fE - rI\end{aligned}\quad (2)$$

Equation system 1 uses gametocyte density data as the input. It is based on the assumption that there is a sequestered pool of gametocytes near maturity (S). Gametocytes in this pool are released from their sequestration sites at a rate of ρ (per day) after treatment with an ACT. The remaining immature gametocytes are destroyed by the ACT. The previously sequestered gametocyte forms enter the peripheral blood circulation and join the pool of mature gametocytes circulating in the peripheral blood (P). Circulating gametocytes are cleared at a rate of μ (per day).

Equation system 2 is the equivalent model at a human population level and uses gametocyte prevalence as the input. The population fraction E , initially gametocyte negative but harboring sequestered gametocytes, progresses into the I (infectious, gametocytemic) state at a constant rate, f . Gametocytemic individuals (I) become gametocyte free at a rate of r . I_0 represents the gametocyte-positive population fraction on the day of enrollment. Therefore, in this model, $1/f$ represents the average time taken for a treated individual to become gametocytemic, and $1/r$ is the average time that an individual carries gametocytes. Since the population fractions E and I are related through $E + I = 1$, E_0 (the fraction of the population that is gametocyte negative but harboring sequestered gametocytes at presentation) can be replaced by $1 - I_0$.

Equation systems 1 and 2 can be solved analytically by integration to

FIG 2 *Plasmodium falciparum* gametocyte density determined by magnetic fractionation for children treated with artemether-lumefantrine or artemisinin-naphthoquine. Equation 3 has been fitted to gametocyte density data (upper panels), and equation 4 has been fitted to gametocyte prevalence data (lower panels). Means (●) and best fits (solid black lines) are shown together with 95% confidence levels (shaded areas). A horizontal dash, representing a gametocyte density of 2.5/μL, is shown in the upper panels.

yield the peripheral gametocyte density over time, $P(t)$, and the peripheral gametocytemia in individuals over time, $I(t)$.

$$P(t) = P_0 e^{-\mu t} + \rho S_0 \frac{e^{-\mu t} - e^{-\rho t}}{\rho - \mu} \quad (3)$$

$$I(t) = I_0 e^{-rt} + f(1 - I_0) \frac{e^{-rt} - e^{-ft}}{f - r} \quad (4)$$

Equation 3 was fitted to gametocyte density data and equation 4 was fitted to gametocyte prevalence data using maximum likelihood methods and based on the following assumptions (4): (i) individual gametocyte clearance times ($1/\mu$) and sequestered gametocyte release times ($1/\rho$) are natural rates that are not affected by the type of ACT (These rates were, therefore, determined from the best fit for the combined data from both treatment arms.), (ii) differences in clearance kinetics originate from differences in the mass of sequestered gametocytes prior to treatment, and (iii) parameter S_0 is not small relative to the concomitant mass of blood stage gametocytes. In relation to (iii), S_0 was chosen to be small (approximately 1/μL for ACT, the same as on day 3) in the study by Bousema et al. (4) based on the assumption that ACT destroys most of the sequestered gametocyte population, limiting the contribution of the first equation in system 1, above. Given the initial rise in gametocyte density observed in both treatment arms in the present study (more pronounced after artemisinin-naphthoquine; see Fig. 2), this assumption appeared invalid, and we therefore allowed this parameter to vary freely.

Statistical analysis. Unless otherwise stated, data are presented as medians and (interquartile ranges [IQRs]). Comparisons between proportions used the χ^2 test, and the Mann-Whitney U test was used for between-group comparisons of continuous variables. All P values are two tailed and unadjusted for multiple comparisons.

RESULTS

Of the 70 children enrolled, 54 were infected with *P. falciparum*, 14 with *P. vivax*, and 2 with both *P. falciparum* and *P. vivax*. Of those with *P. falciparum*, 79% ($n = 23$) were gametocyte positive at enrollment in the artemether-lumefantrine arm, and 84% ($n = 21$) were gametocyte positive in the artemisinin-naphthoquine arm. These values correspond to I_0 in equation 4. All children with *P. vivax* infection also had detectable *P. vivax* gametocytes. The baseline characteristics of the patients and their malaria-related parameters at study entry are summarized in Table 1. There were no statistically significant differences between the groups apart from borderline higher *P. vivax* parasite densities at study entry in those treated with artemisinin-naphthoquine and a trend toward higher *P. falciparum* gametocyte densities in this group.

Posttreatment *Plasmodium falciparum* gametocyte clearance. There was an initial rise in the peripheral *P. falciparum* gametocyte density after treatment with both ACTs, but it was more

TABLE 1 Baseline characteristics of the recruited children by allocated treatment

Characteristic	Artemether-lumefantrine (<i>n</i> = 34) ^a	Artemisinin-naphthoquine (<i>n</i> = 36) ^a	<i>P</i>
Age, mo	46 (40–51)	41 (36–46)	0.26
Males	13 (38)	21 (58)	0.17
<i>P. falciparum</i> infections	28 (82)	24 (67)	0.26
<i>P. vivax</i> infections	4 (12)	10 (28)	0.13
Mixed <i>P. falciparum</i> / <i>P. vivax</i> infections	1 (3)	1 (3)	>0.99
<i>P. falciparum</i> parasite density, per μ l	10,557 (5,471–20,373)	18,339 (9,796–34,334)	0.31
<i>P. vivax</i> parasite density, per μ l	1,162 (107–12,541)	7,765 (2,661–22,658)	0.05
<i>P. falciparum</i> gametocyte density, per μ l	41 (12–128)	19 (8–47)	0.07
<i>P. vivax</i> gametocyte density, per μ l	83 (15–458)	62 (22–171)	0.60

^a Data shown are number (%) or median (interquartile range).

pronounced in the artemisinin-naphthoquine group (see Fig. 2). The pooled median (IQR) values for individual gametocyte-sequestered gametocyte release times ($1/\rho$) and gametocyte circulation times ($1/\mu$) were 4.7 and 3.7 days, respectively (see Table 2). A pooled approach for ACTs was adopted in the original model (4). Both of these present values [release and circulation times] lay within previously published ranges (4 to 12 days and 3 to 6 days, respectively) (4, 31, 32). The sequestered gametocyte populations (S_0) resulting from the best fits to the mean curves (see Fig. 2) were 21/ μ l for artemether-lumefantrine and 61/ μ l for artemisinin-naphthoquine ($P < 0.001$; see Table 2). The median time for gametocyte density to fall to $<2.5/\mu$ l ($t_{2.5}$), the threshold below which transmission becomes unlikely (26, 33), was 16 days in the artemether-lumefantrine group and 20 days in artemisinin-naphthoquine-treated patients ($P < 0.001$; see Table 2). Figure 3 shows the relationship between P_0 , S_0 , and $t_{2.5}$ under equation 3. Values of $t_{2.5}$ are more strongly dependent on S_0 than on P_0 , consistent with the differences in mean S_0 and $t_{2.5}$ between the artemether-lumefantrine and artemisinin-naphthoquine treatments observed in the present study.

The results of *P. falciparum* gametocyte prevalence modeling

TABLE 2 Fitted parameters for the model of *P. falciparum* gametocyte clearance

Model and parameter	Artemether-lumefantrine ^a	Artemisinin-naphthoquine ^a	<i>P</i>
Gametocyte density model ^b			
$1/\rho$	4.7 (3.1–7.8) ^c		
$1/\mu$	3.7 (2.7–5.2) ^c		
S_0	21 (4–45)	61 (49–73)	<0.001
$t_{2.5}$	16 (14–19)	20 (19–21)	<0.001
Gametocyte prevalence model ^d			
$1/f$	5.3 (3.8–7.6)	2.2 (1.2–3.4)	<0.001
$1/r$	12.8 (11.5–14.1)	20.1 (18.2–22.1)	<0.001
$t_{50\%}$	8.9 (7.7–10.1)	14.2 (12.9–15.6)	<0.001

^a Data shown are median (interquartile range).

^b In the gametocyte density model, $1/\rho$ represents the median sequestered gametocyte release time per gametocyte; $1/\mu$, the median circulation time per gametocyte; S_0 , the median reservoir of sequestered gametocytes at study entry; and $t_{2.5}$, the median time to a gametocyte density $<2.5/\mu$ l.

^c Determined from the combined data set.

^d In the gametocyte prevalence model, $1/f$ is the median time before an individual becomes gametocytemic; $1/r$, the median time for an individual to remain gametocytemic; and $t_{50\%}$, the time at which 50% of individuals have cleared gametocytes.

suggests that artemisinin-naphthoquine-treated children became gametocytemic faster (2.2 days) than artemether-lumefantrine-treated children (5.3 days; see Table 2), consistent with a larger S_0 in the former group in the gametocyte density model. In addition, there was a longer gametocyte carriage time per individual in the artemisinin-naphthoquine group (20 versus 13 days; see Table 2), also consistent with the gametocyte density model. The time for gametocyte prevalence to decrease to below 50% of its initial value ($t_{50\%}$) was also significantly longer after artemisinin-naphthoquine treatment than artemether-lumefantrine treatment (14 versus 9 days; see Table 2).

Posttreatment *Plasmodium vivax* gametocyte clearance.

Plasmodium vivax exhibited very different gametocyte clearance kinetics compared to those of *P. falciparum*. For both drug regimens, all *P. vivax* gametocytes were cleared within 2 days of treatment (see Fig. 4). A linear model appeared the simplest and most appropriate fit for both *P. vivax* gametocyte density and prevalence data. Although the slope of the decline in gametocyte density was steeper for the artemether-lumefantrine-treated children than the artemisinin-naphthoquine-treated children (-1.5 ± 0.5 versus -0.6 ± 0.3 , respectively), there were low numbers of densities available after initiation of treatment. When gametocyte prevalence was considered, the slopes of the clearance were similar (-0.7 ± 0.1 and -0.4 ± 0.1 , respectively).

Recurrences of gametocytemia during follow-up. Although *P. vivax* gametocytes were cleared much more rapidly than *P. falciparum* gametocytes, the proportion of children who became *P. vivax* gametocyte positive by day 42 was much higher than that observed with *P. falciparum* (see Fig. 5). Nearly 40% of children in the artemether-lumefantrine group developed *P. vivax* gametocytemia between days 28 and 42 compared with only 3% in the artemisinin-naphthoquine group ($P < 0.001$). In contrast, only 10% of children had become *P. falciparum* gametocyte positive by day 42 in the artemether-lumefantrine group versus 3% in the artemisinin-naphthoquine group ($P = 0.35$). These data reflect between-treatment differences in reappearance of sexual parasite forms during follow-up. They are also consistent with the results of the main trial, in which the appearance/reappearance of *P. vivax* asexual parasitemia in a larger number of children than in the present substudy was significantly more common in the artemether-lumefantrine group regardless of whether *P. vivax* or *P. falciparum* was present on the blood smear at presentation (29).

DISCUSSION

The present data, based on sensitive QMF methods for gametocyte quantification (28), extend observations made in the main

FIG 3 Time to reach a gametocyte density of $<2.5/\mu\text{L}$ ($t_{<2.5}$) for best-fit values of μ (0.27) and ρ (0.21) for variable initial peripheral gametocyte density (P_0) and initial sequestered gametocyte density (S_0). The numbers on the contour lines are $t_{<2.5}$. The mean values of P_0 and S_0 for artemether-lumefantrine and artemisinin-naphthoquine are shown.

trial in which *P. falciparum* gametocyte carriage detected by LM was more frequent in artemisinin-naphthoquine-treated children between days 3 and 14, regardless of whether gametocytes were present at study entry (29). We recruited a subset of over a quarter of the total sample to a substudy in which serial sensitive quantitative gametocyte detection (24, 26–28) was used, together with previously published but modified mathematical models of gametocyte clearance (4), to assess between-treatment differences in more detail. The weight of evidence from the modeling suggests that artemisinin-naphthoquine has less activity against young sequestered *P. falciparum* gametocytes than artemether-lumefantrine. As in the main trial (29), the two regimens were both rapidly effective in the initial clearance of *P. vivax* gametocytemia detected by QMF. The substantial late reappearance of *P. vivax* gametocytemia in artemether-lumefantrine-treated children, largely as a result of recurrent asexual parasitemias that were less effectively suppressed because of the relatively short half-life of lumefantrine (29, 34), was also confirmed.

The variable S_0 in the gametocyte density model appeared a key determinant of posttreatment *P. falciparum* gametocyte kinetics in our patients. This variable was given a relatively small value ($1/\mu\text{L}$) in best-fit sensitivity analyses in the original description of the model (4), but we found much higher values ($21/\mu\text{L}$ and $61/\mu\text{L}$ for artemether-lumefantrine and artemisinin-naphthoquine, respectively). This discrepancy may reflect the fact that gametocyte densities were not determined on days 1 or 2 in the original study (4), making the value of S on day 3 (S_3 , also $1/\mu\text{L}$ for ACT) the gametocyte input parameter of primary interest. The clear rise in gametocyte density during the first few days in both of our treatment groups shows that there is an easily detectable release of sequestered gametocytes into the circulation when ACT is started.

FIG 4 *Plasmodium vivax* gametocyte density (upper panel) and gametocyte prevalence (lower panel) following treatment with artemether-lumefantrine (dashed lines between ●) and artemisinin-naphthoquine (solid lines between ■). The dashed lines represent linear regression models fitted to the mean data, with standard errors of the mean (vertical bars).

There are two possible explanations as to why S_0 in the artemisinin-naphthoquine group was approximately three times that in the artemether-lumefantrine group. First, this may have been a chance occurrence, and subsequent changes in peripheral gametocyte density were treatment independent. The sexual and asexual parasite densities in the peripheral blood were greater in the artemisinin-naphthoquine-treated children, albeit not significantly, which might support this possibility. Alternatively, the effects of artemisinin on stage III and IV sequestered gametocytes about to be released into the circulation at study entry may have been relatively weak. Consistent with this hypothesis, other clinical studies have shown that artemether has greater gametocytocidal effects than other artemisinin drugs (14). In addition, there was evidence that artemisinin-naphthoquine-treated children became gametocytemic more rapidly in the gametocyte density model (with a shorter $1/f$), while the average time that an individual carried gametocytes was longer in the artemisinin-naphthoquine group than in the artemether-lumefantrine group.

Given that it is likely (and as assumed in the density model) that the mean (mature) gametocyte circulation time does not vary by ACT, the longer $1/r$ in the prevalence model may reflect a relatively protracted release of sequestered forms into the circulation in artemisinin-naphthoquine-treated children. The auto-induction of artemisinin metabolism and a consequent reduction in plasma artemisinin concentrations during the 3 days of treatment (34, 35) may have contributed to this, especially since artemether is metabolized to active dihydroartemisinin, while artemisinin is not (36). Nevertheless, intrinsic stage III and IV gametocyte drug

FIG 5 Proportions of individuals in each treatment arm who developed either *P. falciparum* (left panel) or *P. vivax* (right panel) gametocytemia between 4 and 6 weeks after treatment. The vertical bars represent 95% confidence intervals.

sensitivity differences may be important, since the area under the plasma artemether-DHA concentration-time curve ($AUC_{0-\infty}$) in PNG children treated with the more effective conventional six doses of artemether-lumefantrine ($5,902 \mu \cdot \text{h/liter}$ [37]) is less than that expected for artemisinin (which is not metabolized to DHA or other active metabolite) in the same population ($>8,327 \mu \cdot \text{h/liter}$ [34]) after the present three-dose artemisinin-naphthoquine regimen.

It has been suggested that the reduction in *P. falciparum* gametocyte density after treatment with ACT at least partly reflects the rapid clearance of asexual forms (38). There were no between-group differences in initial asexual parasite clearance in the main trial (29), so this phenomenon is unlikely to have been influential in the present substudy. In addition, and in support of the validity of our findings, the gametocyte carriage times in the two groups (13 and 20 days in the artemether-lumefantrine and artemisinin-naphthoquine groups, respectively) are in accord with those in studies that have used the same mathematical model (12.8 days after artemether-lumefantrine in Ugandan children ages 1 to 10 years [39], 13.4 days after treatment with sulfadoxine-pyrimethamine plus artesunate [SP-AS] in Kenyan children ages 0.5 to 10 years [4], and 28 days in a young Tanzanian cohort also treated with SP-AS [4]).

The clinical significance of the 4-day difference between the treatment groups in the time to reach a theoretical peripheral gametocyte density transmission threshold of $<2.5/\mu\text{l}$ is likely to depend on local malaria endemicity. In areas with a high entomological inoculation rate, artemether-lumefantrine may reduce the likelihood of posttreatment transmission (40), albeit at the cost of late recurrences of asexual forms, especially *P. vivax* where this species is transmitted (29). Single-dose primaquine kills *P. falciparum* gametocytes and has been proposed as an addition to *P. falciparum* treatment to reduce transmission (39). However, given its potential hemolytic toxicity and questions regarding the most suitable socioeconomic and epidemiologic settings for its use, it is currently not a part of currently recommended therapy for *P. falciparum* infection (41–43).

The different rates of *P. falciparum* and *P. vivax* gametocyte development and clearance may influence the effectiveness of

treatment strategies aimed at reducing transmission. Whereas *P. falciparum* gametocytes develop relatively slowly (8 to 10 days) and persist after nonprimaquine schizonticidal treatment, *P. vivax* gametocytes develop faster and clear at a rate similar to that of asexual stages after treatment (8). It has been hypothesized, therefore, that *P. vivax* is frequently transmitted to the mosquito vector prior to the onset of symptomatic disease, while most *P. falciparum* transmission occurs after successful treatment of asexual forms. An examination of this hypothesis was beyond the scope of the present substudy but, in the case of *P. vivax*, would be best examined in inoculation experiments in healthy volunteers (44).

The QMF assay used in the present study could be used in other contexts. First, it could be used to determine gametocyte sex ratio after treatment. This was not possible in the present study given that logistic constraints meant that there were several hours between sample collection and assay. Although samples were stored and transported at 37.0°C , we cannot exclude the possibility that male gametocytes exflagellated during this time, a limitation of the present study. Second, the QMF assay could be used for gametocyte viability assessments by conducting exflagellation assays after fractionation (45). This would enable a better assessment of the effect of antimalarial drugs on the ability of circulating gametocytes to infect mosquitoes, as it is currently not clear whether all gametocytes in the peripheral blood are viable after treatment (9, 10).

The present study, conducted using a subset of patients participating in a larger-scale intervention trial (29), provides some evidence that artemisinin is less effective at clearing *P. falciparum* gametocytes than artemether when given as part of ACT to Melanesian children with uncomplicated malaria. This evidence reflects the less-potent activity against stage III and IV sequestered forms, which continue to be released during the 3 days of treatment. Based on a threshold of 2.5 gametocytes/ μl , artemisinin-naphthoquine-treated children can transmit *P. falciparum* for 4 days longer than those allocated to artemether-lumefantrine treatment, perhaps because of less exposure to gametocytocidal drug concentrations during the 3 days of artemisinin dosing. However, the long elimination half-life of naphthoquine (34) ensures that late reappearances of sexual and asexual forms of *P.*

falciparum and/or *P. vivax* are less frequent than after artemether-lumefantrine.

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We declare no conflicts of interest.

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