Increased Gametocytemia after Treatment: An Early Parasitological Indicator of Emerging Sulfadoxine-Pyrimethamine Resistance in Falciparum Malaria

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Background. Although malaria treatment aims primarily to eliminate the asexual blood stages that cause illness, reducing the carriage of gametocytes is critical for limiting malaria transmission and the spread of resistance.

Methods. Clinical and parasitological responses to the fixed-dose combination of sulfadoxine and pyrimethamine in patients with uncomplicated falciparum malaria were assessed biannually since implementation of this treatment policy in 1998 in Mpumalanga Province, South Africa.

Results. Despite sustained cure rates of >90% ($P = .14$), the duration of gametocyte carriage increased from 3 to 22 weeks (per 1000 person-weeks) between 1998 and 2002 ($P < .001$). The *dhfr* and *dhps* mutations associated with sulfadoxine-pyrimethamine resistance were the most important drivers of the increased gametocytemia, although these mutations were not associated with increased pretreatment asexual parasite density or slower asexual parasite clearance times. The geometric mean gametocyte duration and area under the gametocyte density time curve (per 1000 person-weeks) were 7.0 weeks and 60.8 gametocytes/μL per week, respectively, among patients with wild-type parasites, compared with 45.4 weeks ($P = .016$) and 1212 gametocytes/μL per week ($P = .014$), respectively, among those with parasites containing 1–5 *dhfr/dhps* mutations.

Conclusions. An increased duration and density of gametocyte carriage after sulfadoxine-pyrimethamine treatment was an early indicator of drug resistance. This increased gametocytemia among patients who have primary infections with drug-resistant *Plasmodium falciparum* fuels the spread of resistance even before treatment failure rates increase significantly.

Morbidity and mortality due to falciparum malaria have increased across sub-Saharan Africa, largely because of widespread resistance to chloroquine and sulfadoxine-pyrimethamine [1, 2]. Although elimination of *Plasmodium falciparum* during its pathogenic asexual stages is pivotal for the successful treatment of individual symptomatic patients, at a population level, reducing the carriage of viable gametocytes is crucial for limiting the transmission of malaria parasites. *P. falciparum* is transmitted from the human host to the mosquito vector during the nonpathogenic sexual (i.e., gametocyte) stage. In falciparum malaria, there is a clear relationship—although variable and modulated by the immune response—between the prevalence, duration, and density of gametocyte carriage in the human host and the trans-

missibility of *P. falciparum* (i.e., the probability that the mosquito is infected) [3]. Effective antimalarial treatment limits gametocyte production by reducing the asexual parasite population that gives rise to the gametocytes and by killing immature gametocytes [4]. Sulfadoxine-pyrimethamine treatment is associated with a greater prevalence and density of posttreatment gametocytemia than quinoline antimalarials [3]. Resistance to antimalarial drugs spreads because of the increased transmission potential of resistant parasites. This has previously been attributed to greater rates of treatment failure and to increased rates of gametocyte carriage associated with recrudescent infections [5].

In South Africa, seasonal, low-intensity transmission of malaria limits the development of partial immunity, simplifying the analysis of the factors associated with increased gametocytemia. The period during which sulfadoxine-pyrimethamine remains useful for the treatment of malaria has been relatively short in most African settings because of the rapid spread of parasite resistance. However, cure rates of >90% over a 42-day follow-up period were sustained in Mpumalanga Province during the 5-year period after 1998, when the fixed-dose combination of sulfadoxine-pyrimethamine became policy for treatment of malaria [6–8]. We compared the therapeutic efficacy of sulfadoxine-pyrimethamine against sexual and asexual stages of *P. falciparum* and explored the factors associated with gametocyte carriage in patients in Mpumalanga Province between 1998 and 2002.

PATIENTS, MATERIALS, AND METHODS

Patients. In vivo therapeutic efficacy studies were conducted during the malaria seasons of 1998, 2000, and 2002 in Mpumalanga Province, an area in which malaria transmission is seasonal and of low intensity (<200 cases of falciparum malaria occur per 1000 population per annum). A rapid diagnostic test that detects *P. falciparum* histidine-rich protein 2 (ICT [SA Scientific]) was used to screen symptomatic patients for *P. falciparum* infection. Patients in whom *P. falciparum* was detected on a thick blood smear were recruited into the study if they satisfied the following modified World Health Organization (WHO) inclusion criteria [9]: age of ≥2 years, asexual parasite density of >1000 parasites/μL, sufficient proximity to the clinic to allow adequate follow-up, presence of fever (or history of fever within 24 h before recruitment), and provision of written informed consent. Exclusion criteria included use of any antimalarial treatment in the preceding 6 weeks, severe malaria, intolerance of oral therapy, and pregnancy. Patients were treated with a standard single oral dose of sulfadoxine-pyrimethamine (Fansidar [Roche]).

The Mpumalanga Department of Health and the University of Cape Town Research Ethics Committees approved the study. Written informed consent in the patient's home language was obtained from each adult patient or from the guardians of each pediatric patient before enrollment. These studies were conducted in accordance with Good Clinical Practice guidelines [18].

Laboratory assessment and outcome measures. Clinical and parasitological assessments were conducted routinely on days 1, 2, 3, 7, 14, 21, 28, and 42 after treatment. Parasite density was measured by counting the number of asexual parasites per 300 leukocytes. During each visit except those on days 1 and 2 after treatment, the number of gametocytes per 1000 leukocytes was calculated on a Giemsa-stained thick blood film; a standard leukocyte count of 7500 cells/μL of blood was assumed. In 1998, gametocyte positivity rates were measured, but densities were not. Response to treatment was assessed according to the latest WHO classification for areas of low-to-moderate transmission [9]. Adequate clinical and parasitological response (ACPR) was defined as the absence of parasitemia on day 42, without previously meeting criteria for early treatment failure or late clinical or parasitological failure.

Blood specimens obtained via finger prick were collected on filter paper (Whatman No. 3 [Merck Laboratory Supplies]) at each follow-up visit and stored individually at room temperature in ziplock packets that contained desiccant. Parasite DNA was extracted from the blood spots [10]. Once a sample was identified as *P. falciparum* positive by nested polymerase chain reaction (PCR) [11], sequence-specific oligonucleotide probes were used to detect known single-nucleotide polymorphisms (SNPs) in PCR-amplified genes encoding dihydrofolate reductase *(dhfr)* and dihydropteroate synthetase *(dhps)* [12]. Each SNP locus was classified as either wild-type (i.e., associated with antimalarial susceptibility) or mutant. PCR amplification of the polymorphic genetic markers MSP1, MSP2, and GLURP1 was used to differentiate between true recrudescence and new infection [13].

Statistical analyses. Statistical significance was measured using the Kruskal-Wallis test for continuous variables and the χ^2 test for categorical variables. Tests for trend were performed only when there was a prior hypothesis of a trend across study years (the ACPR rate was analyzed using the χ^2 test for trend) or mutation category (gametocyte carriage was analyzed using the Cuzick nonparametric test for trend). The parasite reduction ratio was calculated by dividing the pretreatment asexual parasite density by the parasite density 48 h after treatment (or by 50 if the parasite density at 48 h was zero, on the assumption that 50 parasites/μL is the minimum microscopically detectable density), and was analyzed on a $\log_{10}$ scale [14]. Times to parasite clearance and fever clearance were compared using the log-rank test, taking censored observations into account.

As a measure of cumulative transmission potential, gametocyte carriage was summarized as the area under the time curve (AUC) for gametocyte density and for the infectivity index, using the trapezoidal rule. For patients who were gametocyte car-

Table 1. Baseline characteristics of patients in sulfadoxine-pyrimethamine efficacy studies in Mpumalanga Province, South Africa, who were or were not followed up for >3 days, by study year.

Characteristic	Patients followed for >3 days after enrollment				Patients excluded ≤3 days after enrollment
	1998	2000	2002	*P*	
Age, median (IQR), years	15 (10–23)	17 (9–28)	19 (11–28)	.084	24[a] (14–35)
Male sex	57/112 (50.9)	52/108 (48.2)	62/148 (41.9)	.33	21/35 (60)
Asexual parasite density, parasites/μL					
Geometric mean	37,199	22,210	23,251	.0001	33,361
95% confidence interval	33,869–40,857	19,374–25,461	18,949–28,530		23,118–48,141
Pretreatment gametocyte positivity	0/112 (0)	1/108 (0.9)	7/148 (4.7)	.020	1/35 (2.9)
Pretreatment mutation frequency[b]					
Triple	14/93 (15.1)	15/102 (14.7)	26/130 (20.2)	.48	9/27 (33.3)
Double	6/93 (6.5)	5/102 (4.9)	19/130 (14.6)	.022	6/27 (22.2)
Quintuple	5/93 (5.4)	4/102 (3.9)	13/130 (10.0)	.15	4/27 (14.8)

NOTE. Data are proportion (%) of patients who tested positive for *Plasmodium falciparum,* unless otherwise indicated. IQR, interquartile range.

[a] Statistically significant difference between the ages of patients excluded and those included in this study.

[b] Data are for triple mutants (i.e., *P. falciparum* gametocytes with mutations at codons 108, 51, and 59 in *dhfr*), double mutants (those with mutations at codons 437 and 540 in *dhps*), and quintuple mutants (those with the specified mutations in both *dhfr* and *dhps*).

riers, 2.5 days was added to the observed duration of carriage, to account for uncertainty associated with interval-based measurement. The predicted infectivity index was calculated using the gametocyte density at each day of follow-up, which was based on the log sigmoid relationship between gametocyte density and infectivity observed previously in nonimmune patients [3]. This assumed no interindividual variability in the relationship between gametocyte density and infectivity. Informative censoring occurred, as gametocyte carriage was not measured after study withdrawal due to treatment failure. To limit this effect, the duration of gametocyte carriage and the gametocyte and infectivity index AUCs were expressed per 1000 person-weeks of follow-up, dividing the value measured by the number of weeks of follow-up and multiplying by 1000 [5]. These values were summarized as geometric means (95% confidence intervals [CIs]) after addition of 0.1 to the observed value to ensure that densities of zero, which are important for limiting malaria transmission and the spread of resistance, were included in this summary measurement.

Gametocyte positivity rates and densities were also analyzed in a longitudinal framework to model the gametocyte density–time profile over 6 weeks. As gametocyte density data were characterized by excess zeroes (i.e., by visits in which no gametocytes were detected) and overdispersion, we used the zero-inflated negative binomial model, a mixture model that models prevalence by use of logistic regression and density by use of negative binomial regression, to analyze gametocyte carriage in 2000 and 2002. These models generated parallel estimates of the risk ratio

(RR) of gametocyte positivity and the incidence rate ratio (IRR) of the mean gametocyte densities [15–17]. We accounted for within-subject correlation by use of robust estimates of SEs. Study year, baseline asexual parasite density ($\log_{10}$ transformed), fever, age, dose, sex, fever, parasite clearance time, treatment failure, and frequencies of *dhfr* and *dhps* mutations were defined prospectively as potential risk factors for increased gametocyte carriage.

RESULTS

Study population. In total, 403 patients were recruited in the 3 open-label studies: 132 patients entered in 1998, 119 in 2000, and 152 in 2002. Because there is a lag time of approximately three 48-h asexual life cycles between peak asexual parasitemia and gametocytemia [19], our analysis was confined to patients followed for >3 days (which was long enough to measure gametocyte carriage). Thirty-five patients (8.7%) were lost to follow-up or withdrawn from the study by day 3. Baseline characteristics of the remaining 368 patients and the excluded patients are summarized in table 1. The pretreatment geometric mean asexual parasite density was significantly larger in 1998 than in 2000 and 2002 ($P < .001$). More patients carried gametocytes before treatment (day 0) in 2002 than in previous years ($P = .02$). The excluded patients were significantly older than those who remained in the study ($P = .016$). Only 5 (1.4%) of the 368 patients who remained in the study were subsequently lost to follow-up.

Table 2. Effect of pretreatment *Plasmodium falciparum dhfr* and/or *dhps* mutations on asexual parasite density among patients in sulfadoxine-pyrimethamine efficacy studies in Mpumalanga Province, South Africa, in 1998, 2000, and 2002 who were followed up for >3 days.

Variable	Wild-type genotype	1–4 mutations	Quintuple mutation	*P*
Baseline asexual parasite density, GM (95% CI), parasites/μL	24,629 (21,609–28,072)	26,673 (21,754–32,703)	30,057 (20,878–43,270)	.38[a]
Parasite clearance time, median (IQR), days	2 (2–3)	2 (2–3)	2 (2–3)	.49[b]
Log$_{10}$ parasite reduction ratio, median (IQR)	2.45 (1.51–2.71)	2.26 (1.37–2.76)	2.30 (1.81–2.91)	.92[a]

NOTE. CI, confidence interval; GM, geometric mean; IQR, interquartile range.
[a] By the Kruskal-Wallis test.
[b] By the log-rank test.

dhfr and dhps genotypes. Pretreatment *dhfr* and *dhps* mutations could be defined in 325 patients (88.3%); samples from 7 patients were missing, and DNA could not be extracted from samples from 36 patients. Although the fully susceptible wild-type genotypes predominated throughout the 5-year period, there was a significant increase in the frequency of *dhps* double mutations (at codons 437 and 540), from 4.9% (5 of 102 patients) in 2000 to 14.6% (19 of 130) in 2002 (RR, 2.98; 95% CI, 1.15–7.71; *P* = .016). Without exception, the *dhps* mutations at codon 437 preceded that at codon 540, which only occurred in patients with the quintuple mutations (defined as the presence of *dhfr* triple mutations [at codons 108, 51, and 59] and *dhps* double mutations). The increases in the frequency of pretreatment *dhfr* triple mutations and quintuple mutations were not statistically significant (table 1). The pretreatment geometric mean asexual parasite density was similar for all pretreatment genotypes (*P* = .38) (table 2).

Clinical and parasitological responses. The monotonic trend toward increased treatment failure rates during the 5 years after sulfadoxine-pyrimethamine introduction was not statistically significant (*P* = .14). Of the 147 patients (96.7%) who completed the study in 2002, a total of 134 (91%) had an adequate ACPR. This cure rate was similar to that observed in 2000 (96.3% [104 of 108 patients]; *P* = .10) and 1998 (95.4% [103 of 108]; *P* = .19). The median parasite clearance times were more rapid in 1998 and 2000 (2.0 days in both years) than that in 2002 (3 days; *P* < .0001 by the log-rank test). Similarly, the parasite reduction ratio decreased significantly from almost a 1000-fold reduction per 48-h asexual life cycle (log$_{10}$ reduction ratio, 2.74) in 1998 to approximately a 100-fold reduction per 48-h asexual life cycle (log$_{10}$ reduction ratio, 1.96) in 2002 (*P* < .001). Parasite clearance times were <48 h in 216 (59.5%) of 368 patients overall, decreasing from 79.6% in 1998 to 50.3% in 2002. The median fever clearance time remained 3 days from 1998 to 2002.

The risk of treatment failure was strongly associated with *dhfr* and *dhps* genotypes (*P* < .001); the cure rate (i.e., ACPR) was 99.6% (263 of 264 patients) for infections involving parasites with <3 mutations, 96.9% (31 of 32) for those involving parasites with 3 mutations, and only 25.0% (1 of 4) and 22.7% (5 of 22) for those involving parasites with 4 and 5 mutations, respec-

tively (figure 1). Thus, the RR of treatment failure for infections involving parasites with ≥4 mutations was 4-fold higher (RR, 4.3; 95% CI, 2.13–8.68; *P* < .001) than for those involving parasites with fewer mutations. No association was seen between mutation frequency and either asexual parasite reduction ratio (*P* = .92) or parasite clearance time during the primary infection (*P* = .49 by the log-rank test) (table 2).

Gametocyte carriage between 1998 and 2002. Gametocytemia data were missing for 238 (6.6%) of 3627 blood smears. Gametocyte positivity rates (i.e., the proportion of patients with patent gametocytemia) for all study patients without gametocytes before treatment showed that positivity rates peaked consistently on day 14 and increased significantly between 1998 and 2002 (*P* = .008 by the Fisher exact test) (figure 2). Gametocyte densities, which were only determined in 2000 and 2002, peaked on day 14 in both years.

Gametocyte carriage was analyzed further after excluding 8 patients who had gametocytemia before treatment (table 3). The percentage of patients with posttreatment gametocytemia increased significantly, from 35% (39 of 112 patients) in 1998 to
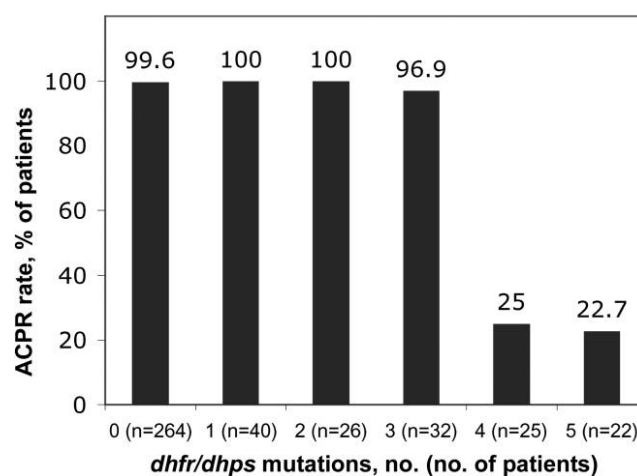

Figure 1. Association between the 42-day adequate clinical and parasitological response (ACPR) rate and the number of mutations in the *dhfr* and/or *dhps* genes in sulfadoxine-pyrimethamine efficacy studies in Mpumalanga Province, South Africa, in 1998, 2000, and 2002.
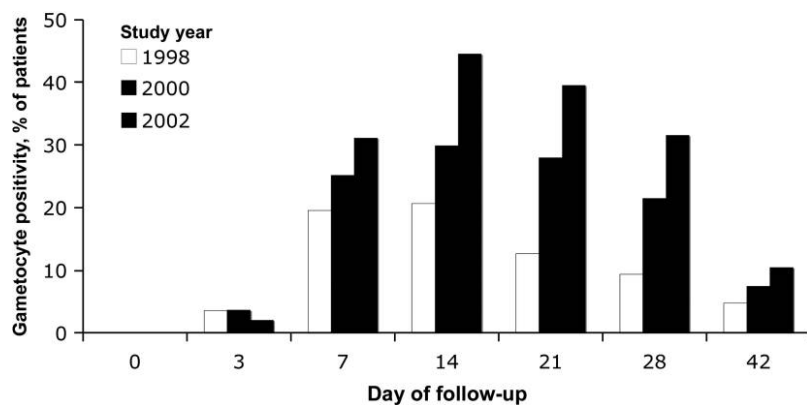
Figure 2. Rates of *Plasmodium falciparum* gametocyte positivity among patients without pretreatment gametocytes who were enrolled in sulfadoxine-pyrimethamine efficacy studies in Mpumalanga Province, South Africa, by day of follow-up and study year.

53% (75 of 141) in 2002 ($P = .013$), with significant increases in gametocyte positivity rates seen on days 14 and 21 ($P < .001$). The geometric mean duration of patent gametocytemia (per 1000 person-weeks) increased between 1998 and 2002, with durations of 3 weeks in 1998, 11 weeks in 2000, and 22 weeks in 2002 ($P < .001$). The mean maximum gametocyte densities increased 7-fold, from 80 gametocytes/μL in 2000 to 567 gametocytes/μL in 2002 ($P < .001$). There was a 6-fold increase in the geometric mean gametocyte AUC, from 79 gametocytes/μL per week in 2000 to 471 gametocytes/μL per week in 2002 ($P = .006$). The geometric mean infectivity index AUC increased >3-fold, from 47 infectivity-weeks in 2000 to 171 infectivity-weeks in 2002 ($P = .013$).

Factors associated with gametocyte carriage. Among all patients enrolled in 2000 and 2002, excluding the 8 who were carrying gametocytes before treatment, those infected with resistant parasites were significantly more likely to carry gametocytes. The geometric mean duration of gametocyte carriage was 7 weeks (95% CI, 3–17 weeks) among 133 patients infected with wild-type parasites and 45 weeks (95% CI, 16–126 weeks)

among 91 patients infected with parasites with 1–5 *dhfr/dhps* mutations ($P = .016$). The geometric mean gametocyte AUC was 61 gametocytes/μL per week (95% CI, 16–229) for wild-type infections, compared with 1212 (95% CI, 250–5889) gametocytes/μL per week for infections with any mutants ($P = .014$). There was a corresponding increase in the geometric mean infectivity index AUC, from 31 infectivity-weeks (95% CI, 9–99) to 470 infectivity-weeks (95% CI, 115–1920) ($P = .010$).

To better understand the spread of resistance at a population level, further analyses (which used the Cuzick nonparametric test for trend) included the 8 patients with gametocytes detected before treatment. Patients with parasites bearing the *dhfr/dhps* quintuple mutation had a significantly longer duration of gametocyte carriage ($P = .016$), a larger gametocyte AUC ($P = .022$), and a larger infectivity index AUC ($P = .016$), compared with patients carrying parasites with 1–4 mutations or no mutations (figure 3).

After adjustment for longitudinal trends between follow-up days 3 and 42 and over the 2000 and 2002 study years, multivariate analysis showed that pretreatment asexual parasite density,

Table 3. Characteristics of posttreatment *Plasmodium falciparum* gametocytemia among patients in sulfadoxine-pyrimethamine efficacy studies in Mpumalanga Province, South Africa, by study year.

Variable	1998	2000	2002	*P*
Gametocyte positivity after treatment				
Overall (days 3–42)	39/112 (34.8)	51/107 (47.7)	75/141 (53.2)	.013
Day 7	22/112 (19.6)	27/107 (25.2)	44/141 (31.2)	.11
Day 14	23/111 (20.7)	32/107 (29.9)	62/139 (44.6)	<.001
Day 21	14/110 (12.7)	30/107 (28.0)	55/139 (39.6)	<.001
Maximum gametocyte density, mean (95% CI), parasites/μL	ND	80 (36–124)	567 (336–798)	<.001
Duration of gametocyte carriage, weeks[a]	2.8 (1.2–6.5)	10.8 (4.2–28.0)	21.6 (9.3–50.5)	<.001
Gametocyte AUC, gametocytes/μL per week[a]	ND	79 (19–324)	471 (123–1799)	.006
Infectivity index AUC, infectivity-weeks[a]	ND	47 (13–171)	171 (53–553)	.013

NOTE. AUC, area under the time curve; CI, confidence interval; ND, not determined.

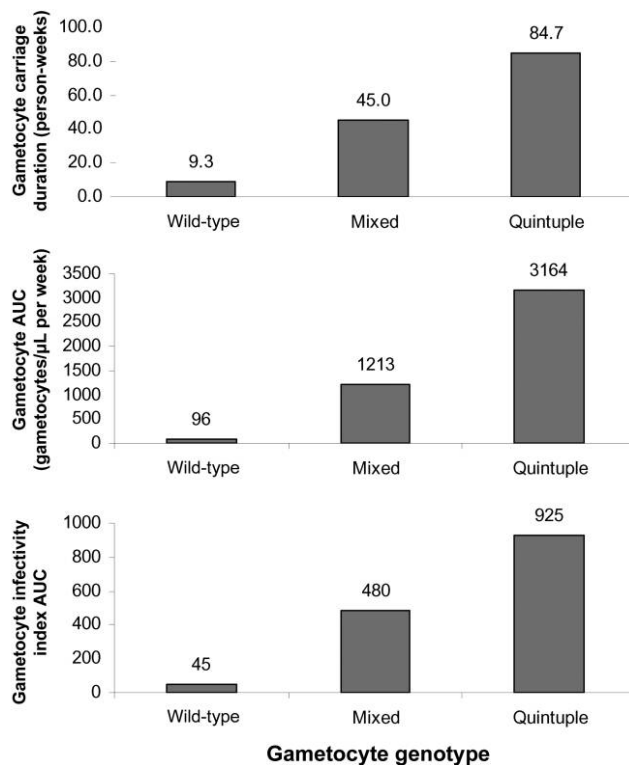[a] Data are geometric mean (95% CI) per 1000 person-weeks of follow-up.

Figure 3. Characteristics of *Plasmodium falciparum* gametocyte carriage per 1000 person-weeks of follow-up among 139 patients carrying parasites with wild-type pretreatment *dhfr* and *dhps* genotypes (i.e., 0 mutations), 76 carrying parasites with "mixed" pretreatment *dhfr* and *dhps* genotypes (i.e., 1–4 mutations), and 17 carrying parasites with a quintuple pretreatment genotype (i.e., 3 mutations in *dhfr* and 2 mutations in *dhps*) who were in a sulfadoxine-pyrimethamine efficacy study in Mpumalanga Province, South Africa, in 2000 or 2002. AUC, area under the time curve.

parasite clearance time, the quintuple *dhfr/dhps* mutation, age, and presence of fever at baseline were associated independently with gametocyte carriage ($P < .05$), although only pretreatment asexual parasite density was significantly associated with both

gametocyte positivity rates and gametocyte density (table 4). The effect of the resistant parasites with quintuple genotype on the mean gametocyte density varied according to the time after treatment. On day 3, gametocyte densities were 6-fold higher (IRR, 6.0; 95% CI, 1.5–24.4; $P = .012$) among patients infected with parasites bearing the quintuple mutation, compared with those infected with parasites carrying fewer mutations (table 4). The lack of a statistically significant association thereafter is likely to reflect the small number of patients with parasites carrying the quintuple mutation ($n = 17$) and informative censoring, because 82% of these patients were withdrawn because of treatment failure.

For every 10-fold increase in pretreatment asexual parasite density, the RR of carrying gametocytes doubled (OR, 2.35; 95% CI, 1.41–3.92) and was accompanied by a similar relative increase in gametocyte density (IRR, 2.15; 95% CI, 1.39–3.32) among patients who were gametocytemic. For each additional day of delay in parasite clearance, there was a 19% increase (OR, 1.19; 95% CI, 1.06–1.33) in the RR of carrying gametocytes. There was no association between parasite mutations and parasite clearance time ($P = .49$ by the log-rank test; table 2).

Increasing age was associated with a slight increase in gametocyte density, with a 16% relative increase for every decade increase in age. Patients who were febrile at enrollment had a greater gametocyte density (IRR, 1.63; 95% CI, 1.04–2.56) than patients with only a history of fever. There was no association between gametocyte positivity rates or density and sex, fever clearance time, or sulfadoxine-pyrimethamine dose. Even after adjusting for prospectively defined risk factors, gametocyte density was significantly greater in 2002 than in 2000 (IRR, 5.22; 95% CI, 3.03–9.00). No association was found between the duration or density of gametocytemia and treatment failure in this study.

DISCUSSION

Sulfadoxine-pyrimethamine remains one of the most widely used antimalarial drugs in the world, both for treatment of

Table 4. Findings of regression analysis of prospectively defined risk factors for *Plasmodium falciparum* gametocyte carriage during days 3–42 of follow-up for patients in sulfadoxine-pyrimethamine efficacy studies in Mpumalanga Province, South Africa, in 2000 and 2002.

Variable	Gametocyte positivity OR (95% CI)	Gametocyte density IRR (95% CI)	Infectivity index IRR (95% CI)
Study year, 2002 vs. 2000	1.61 (0.98–2.65)	5.22 (3.03–9.00)	1.62 (1.40–1.86)
Quintuple mutation[a]	2.15 (0.42–11.11)	6.02 (1.48–24.42)	1.49 (1.00–2.22)
Asexual parasite density, per $\log_{10}$ parasites/μL increase	2.35 (1.41–3.92)	2.15 (1.39–3.32)	1.27 (1.13–1.42)
Time to parasite clearance, per additional day of carriage	1.19 (1.06–1.33)	0.91 (0.81–1.01)	0.99 (0.97–1.02)
Age, per decade increase	0.93 (0.82–1.05)	1.16 (1.00–1.33)	1.03 (0.99–1.07)
Baseline fever	0.83 (0.44–1.56)	1.63 (1.04–2.56)	1.07 (0.94–1.21)

NOTE. Data were obtained via zero-inflated negative binomial regression analysis, with adjustment for follow-up day. CI, confidence interval; IRR, incidence rate ratio; OR, odds ratio.

[a] Interactions between pretreatment presence of the quintuple mutations (triple *dhfr* and double *dhps* mutations) and all days of follow-up were included in the model, although only the effect of the quintuple mutation on day 3, before informative censoring, is shown.

symptomatic patients and intermittent preventive treatment of high-risk groups (pregnant women and infants). Unfortunately, the resistance to sulfadoxine-pyrimethamine that arose from extremely rare mutations has spread rapidly, and in many areas it is no longer effective [20, 21]. Mpumalanga Province was one of the first areas in Africa where policy dictated that sulfadoxine-pyrimethamine be used to treat malaria. Our studies' detailed monitoring of gametocyte carriage (i.e., positivity rates, duration, and density) to predict infectivity provided an accurate description of temporal changes in gametocyte carriage and the factors that influence it. We showed a marked increase in the duration and density of gametocyte carriage over the 5 years since SP was introduced, whereas the monotonic trend toward a decrease in the cure rate was not yet statistically significant. Thus, increasing gametocytemia was a harbinger of increasing clinical resistance.

The low intensity and seasonal malaria transmission in Mpumalanga Province is associated with minimal acquisition of immunity in the local population, which simplified our evaluation of factors affecting gametocyte carriage [22, 23]. It has been thought that the engine driving the spread of drug resistance in areas with a prevalence of resistance is the increased duration of gametocytemia due to recrudescent infections. But our principal finding of a differential increase in carriage of gametocytes in infections with 1–5 *dhfr/dhps* mutations in primary infections, even before treatment failure rates increased significantly, is likely to be a major factor driving the initial rapid migration of resistant genes. Routine measurement of gametocyte carriage during in vivo therapeutic efficacy studies can be used as an early indicator of sulfadoxine-pyrimethamine resistance and transmission potential. The infectivity index we used serves only as a guide to the shape of the relationship between gametocyte density and infectivity and would need to be recalibrated for other populations. Monitoring gametocyte density enabled more sensitive evaluation of the effect of mutations, as the area under the gametocyte density time curve increased 20-fold, whereas the duration of gametocyte carriage only increased 6-fold, when at least 1 *dhfr/dhps* mutation was present. Mendez et al. [24] previously found that the *dhfr* double mutation (at codons 108 and 51) was associated with an increase in gametocyte positivity rates in Colombia, despite high rates of cure after sulfadoxine-pyrimethamine treatment.

This effect of *dhfr* and *dhps* mutations on gametocyte carriage during the primary infection may underestimate the differential effect on transmissibility and, thus, the spread of sulfadoxine-pyrimethamine resistance, because gametocyte viability in the mosquito vector and subsequent sporozoite development is lower (i.e., the sporontocidal effect is greater) in parasites susceptible to sulfadoxine-pyrimethamine, compared with those that are resistant [25]. Once these mutations lead to treatment failure, gametocyte carriage of parasites with resistance-conferring mutations is further increased during recrudescent

infections, which are known to be associated with increases in gametocytemia [5]. Bousema et al. [26] found that posttreatment gametocyte positivity rates were 4.1-fold higher among the 22% of patients who did not respond to sulfadoxine-pyrimethamine treatment within 7 days than among patients who were cured.

The prompt initiation of treatment, immigration of infecting pathogens from areas with less sulfadoxine-pyrimethamine drug pressure, and use of quinine to manage treatment failures in Mpumalanga Province may explain why sulfadoxine-pyrimethamine cure rates had not decreased significantly after 5 years of use as first-line treatment. After adjustment for the *dhfr/dhps* quintuple resistance haplotype, we found that increased gametocyte carriage was associated with greater pretreatment asexual parasite densities and longer parasite clearance times, which are consistent with findings from previous studies [24, 27–30], although most of these studies described the positivity rates and duration, but not density, of gametocyte carriage. Because neither asexual parasite density nor asexual parasite clearance time were associated with the *dhfr/dhps* mutations in our study, the increased duration of carriage of gametocytes with more resistance-conferring mutations did not result from greater levels of asexual parasitemia. Indeed, duration of gametocyte carriage increased markedly between 1998 and 2002, despite a significant decrease in pretreatment asexual parasite density. This implies that, in patients infected with parasites carrying resistance-conferring mutations, treatment with sulfadoxine-pyrimethamine increases rates of switching from asexual to gametocyte stages. With the exception of pretreatment asexual parasite density factors triggering the initial switch from asexual to sexual stage development (i.e., factors associated with gametocyte positivity rates) were not the same as those associated with increased gametocyte density.

Because the public sector is the principal source of antimalarial drugs in South Africa, it is unlikely that other antimalarial drugs were being used in this rural area. There were also no obvious demographic or epidemiological explanations for the increase in gametocyte carriage, other than increasing drug resistance. However, the temporal changes in gametocyte carriage were not fully explained by the factors that we determined. Previous studies have found longer gametocyte carriage in anemic patients, afebrile patients, and patients with a prolonged duration of symptoms [27–30]. In the 3 studies reported here, duration of symptoms was not systematically recorded, and there were relatively few anemic and afebrile patients.

The lowest posttherapy rates of gametocyte carriage follow treatment with artemisinin derivatives or primaquine [3]. Artemisinin-based combination therapies (ACTs) are currently considered the best treatment for uncomplicated falciparum malaria [31]. Their ability to reduce gametocyte carriage [5, 25, 32, 33], and thereby decrease malaria transmission, is one of their key benefits. The decreased gametocyte carriage seen with

widespread use of effective ACTs has been shown to translate into dramatic reductions in malaria transmission in northwestern Thailand and KwaZulu Natal, South Africa—both of which have low intensities of malaria transmission similar to that in Mpumalanga Province—as well as in Zanzibar, an island with historically greater malaria transmission intensity [17, 34, 35]. Importantly, ACTs also decrease the transmission advantage of resistant parasites [5, 36, 37]. Deployment of ACTs was associated with a decrease in mefloquine resistance along the northwestern border of Thailand [34, 36]. By analogy, to ensure optimal malaria control, sulfadoxine-pyrimethamine should be combined with artesunate in areas where sulfadoxine-pyrimethamine remains highly effective.

As a result of the alarming increase in gametocyte carriage observed following sulfadoxine-pyrimethamine treatment between 1998 and 2002, together with the established advantages of artemisinin-based combinations in improving cure rates and potentially delaying resistance, the combined use artesunate plus sulfadoxine-pyrimethamine became policy for treatment of uncomplicated malaria in public sector health care facilities in Mpumalanga Province in January 2003.

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