

## ASSOCIATION OF SUBTHERAPEUTIC DOSAGES OF A STANDARD DRUG REGIMEN WITH FAILURES IN PREVENTING RELAPSES OF VIVAX MALARIA

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**Abstract.** This study evaluated the cure rate of the standard recommended regimen for *Plasmodium vivax* malaria in Brazil and assessed risk factors for failures. Fifty patients with vivax malaria given supervised medical treatment (standard dose of chloroquine: total dose = 1.5 g over a three-day period plus primaquine: total dose = 210 mg over a 14-day period) were followed for six months in a non-endemic area. Cox's regression was used to identify predictors of relapses. Among the 289 patient-months of follow-up, seven relapses were identified (2.4 relapses per 100 person-months) between 33 and 137 days after treatment initiation. Risk factors for relapses ( $P \leq 0.05$ ) were female sex, higher parasitemia at baseline, shorter number of days with symptoms prior to baseline, and lower mg/kg dose of primaquine. Relapses following supervised vivax treatment is in principle a necessary, but not sufficient, component of *in vivo* parasite resistance. Results indicate that other factors, principally sub-therapeutic primaquine doses, may explain the occurrence of vivax treatment failures.

### INTRODUCTION

Relapses of *Plasmodium vivax* malaria due to primaquine treatment failure have been documented since 1950, with few small-scale studies recently reported from Latin America.<sup>1–5</sup> The observation of *P. vivax* relapses that occur later than 28 days after the start of treatment (usually supervised) is generally attributed to parasite resistance to primaquine without further investigation of other determinants.

The recommended treatment for radical cure of adults with vivax malaria is a combination of chloroquine (total dose = 25 mg of base/kg over a three-day period) and primaquine (0.25–0.3 mg/kg per day over a 14-day period, total dose = 3.5–4.2 mg/kg), with primaquine given for its hypnozoitocidal activity.<sup>6,7</sup> Operationally, however, treatment for vivax malaria is typically prescribed for adults according to a weight-independent recommendation (chloroquine: total dose = 1.5 g over a three-day period plus primaquine: 15 mg/day over a 14-day period, total dose = 210 mg).<sup>6</sup> This standard treatment recommendation implies that only patients weighing 50–60 kg or less receive the mg/kg recommended therapeutic dose of primaquine (3.5–4.2 mg/kg). Although some have stressed the importance of the weight-dependent dose, recent studies investigating radical cure rates seldom consider a patient's weight to be a contributing factor to failure.<sup>8</sup> Today, most malaria patients in Brazil are male, usually miners or agriculture workers, with many exceeding this 50–60 kg weight limit, who are prescribed doses of primaquine independent of their weights.

Moreover, there are a number of other factors that may affect the vivax cure rate in a given area, such as patient's level of immunity, parasite strain, and number of parasites inoculated.<sup>9</sup> Although several recent studies have shown failure of the standard vivax treatment, no studies have assessed associations between the likelihood of treatment failure and epidemiologic factors in this context.

Therefore, the objective of this study was to evaluate the cure rate of the recommended treatment regimen for radical cure of vivax malaria in Brazil and to assess risk factors of failures.

### MATERIALS AND METHODS

**Study design.** The project was designed as a clinical trial without control group (prospective open trial) conducted in a non-endemic area, where one group of patients with vivax malaria received standard treatment with supervised administration of all doses of medication. They were then monitored for at least six months (or while they remained in the study) for relapse.

**Population and eligibility criteria.** The study was conducted in the city of Cuiabá, the capital of Mato Grosso state, located in the southern part of the Brazilian Amazon. The city itself has no malaria transmission, but receives malaria patients from several surrounding endemic areas. The study was conducted at the Hospital of the Federal University of Mato Grosso with additional patients recruited from other malaria clinics in the city. These malaria laboratories are open on a daily basis and offer malaria diagnosis and treatment free of charge throughout the year. Admission criteria were a minimum age of 14 years, a diagnosis of vivax malaria without other *Plasmodium* species, no previous malaria treatment for the current malaria episode in the past 15 days, a willingness to stay in Cuiabá for at least six months after starting treatment, and signed informed consent. Appropriate informed consent was obtained and the research was conducted in accordance with guidelines for human research as specified by the Brazilian National Council of Health (Health Ministry, Resolution No. 196/96).

Exclusion criteria were post-admission diagnosis of mixed infections, occurrence of side effects during treatment, or patient refusal to continue with study follow-up.

**Baseline data.** After study inclusion, patients were interviewed using a standard form for information regarding age, sex, weight, pregnancy, days ill prior to admission, symptoms, probable locale of infections, history of malaria, any medication during last 30 days, and use of primaquine. Standard clinical examinations were performed. Spleen size was measured in a supine position as centimeters below the costal margin in the mid-clavicular line. In addition, malaria smears were collected to confirm smear results from the referral clinic, as well as to determine parasite density.

**Laboratory procedures.** Giemsa-stained malaria smears were examined. Briefly, two thick blood smears were prepared for each reading and air-dried. Smears were de-hemoglobinized with methylene blue and stained with Giemsa's stain. Well-trained laboratory technicians examined 100 high-power microscopic fields for parasite identification and quantification. Results were reported as the number of parasites per 200 white blood cells (it was assumed that white blood cell counts were the same among patients).

**Treatment.** This study used the standard dose of chloroquine (3 days: 600 mg on the first day and 450 mg on the second and third days, total dose = 1.5 g) plus primaquine (15 mg/day over a 14-day period, total dose = 210 mg) according to national guidelines. Although each patient received the same amount of primaquine, the dose per kilogram of body weight was calculated, and the association of this variable with relapses was assessed.

**Follow-up data and procedures.** *During treatment.* Investigators supervised administration of all doses either at the patient's home or during his or her follow-up visits to the laboratory. When this procedure was not possible, patients were asked on a daily basis if he or she took all doses of the medication. Moreover, throughout treatment, patients were asked about symptoms and any non-study medications used. They were asked specifically about the occurrence of diarrhea or vomiting after each previous medication dose. If vomiting occurred within 1 hr of medication administration, that medication dose was not "counted" and chloroquine and/or primaquine treatment was extended for one additional dose. Malaria smears were examined on day 1 (baseline/start of treatment) then every other day, until two consecutive negative smears were obtained. Although tests for glucose-6-phosphate dehydrogenase were not performed, most patients reported use of primaquine without signs of hemolysis or other side effects. Because daily visits were planned, side effects due to study medications could be detected promptly, so that treatment could be discontinued.

*After treatment.* After treatment (and parasite clearance), patients were visited monthly and malaria smears were prepared and examined on days 30, 60, 90, 120, 150, and 180 after treatment initiation. Between scheduled visits, patients were instructed to contact investigators for recurrence of any malaria symptoms or if they decided to travel out of the study area. Relapse (or treatment failure) was defined as any reappearance of vivax parasitemia during the follow-up period after completion of treatment and parasite clearance. If patients relapsed, they were re-treated with chloroquine (3 days: total dose = 1.5 g) and a double daily dose of primaquine (30 mg/day over a 14-day period, total dose = 420 mg). Side effects were also monitored during this period. After re-treatment, patients were asked to return to the malaria clinic or to call one of the investigators due to any reappearance of malaria symptoms.

**Statistical analysis.** Data were analyzed using STATA Statistical Software release 5.0.<sup>10</sup> Descriptions of baseline and follow-up data are reported as the mean  $\pm$  SD and proportions. Whenever possible, continuous variables were categorized using biologically meaningful categories. The life table method was used to describe survival curves and to visually compare non-failure rates among different groups. Cox's proportional hazards regression was used to identify

independent predictors of relapses.<sup>11</sup> Time to relapse after baseline was defined as number of days between the baseline visit and the recurrence of a positive vivax smear. Dates (day, month, and year) were recorded for all the following events: baseline examination, parasite clearance and recurrent parasitemia, all study visits, and malaria smear examinations. If an individual did not relapse during follow-up or if an individual was lost to follow-up before experiencing any malaria relapse, for the purpose of survival analyses, this individual was censored at the time of his or her last follow-up visit with a malaria smear examination. Assessment of potential predictors and relapse rate was based on an automatic model selection procedure (backward exclusion criteria  $P > 0.10$ ). The significance level for all hypothesis testing was set at  $P \leq 0.05$ . For the final model using Cox's proportional hazards regression, the proportional hazard assumption was assessed using the method proposed by Grambsch and Therneau, which is incorporated in STATA Statistical Software: release 6.0.<sup>10-12</sup> The rejection of the null hypothesis ( $P < 0.05$ ) indicates deviation from the proportional hazard assumption.

## RESULTS

A total of 56 patients met the inclusion criteria during the study entry period (from February 1997 to October 1998). Six patients (10.7%) were excluded from this analysis due to an initial misdiagnosis, lost to follow-up before treatment completion, or refusal to continue.

The average follow-up period of the 50 patients who remained in the study was 5.8 months (174 days, range = 26-365 days). Most patients (72.0%) were followed for six months or more (Table 1). A total of 8,681 patient-days (or 289.4 patient-months) of follow-up was observed.

The majority of the subjects were male (76.0%) with ages between 14 and 77 years (mean  $\pm$  SD = 31.8  $\pm$  12.8 years) and weights between 42 and 102 kg (mean  $\pm$  SD = 67.3  $\pm$  14.7 kg). They acquired their malaria infections in the Brazilian states of Mato Grosso (54.0%) or Rondonia (24.0%). Use of primaquine was reported by 68.0% of the patients. Few patients ( $n = 3$ ) reported primaquine use from 19 to 30 days prior to study admission. All others reported most recent use of primaquine at least 45 days prior to study admission. Splenomegaly was observed in 19 (40.4%) patients. There was a large variation in the number of clinical malaria episodes reported (mean  $\pm$  SD = 5.8  $\pm$  10.0 episodes). On admission, most patients reported fever (100%), headache (92.0%), and myalgia (64.0%). They were symptomatic a mean  $\pm$  SD of 6.5  $\pm$  4.7 days prior to admission and their mean  $\pm$  SD parasitemia at baseline was 234.4  $\pm$  229.3 parasites per 200 white blood cells.

Among the patients admitted to the study, 18% self-administered medications without supervision and the investigators supervised administration of all doses in the other 82%. A standard 15-mg daily dose of primaquine was prescribed for all patients, and the mean  $\pm$  SD total mg/kg of body weight dose of primaquine administered was 3.26  $\pm$  0.68 mg/kg. Due to the weight distribution of the study population, 30 (62.5%) patients received total doses of primaquine less than 3.5 mg/kg of body weight, 13 (27.1%) patients received total doses of between 3.5 and < 4.2 mg/kg

TABLE 1

Description of the study population and individuals who relapsed following treatment of vivax malaria with primaquine (14 days) and chloroquine (3 days)

Variable (range if continuous)	Category	Study population (n = 50)	Patients who relapsed (n = 7)
Days of follow-up (14–240 days)	<180 days	14 (28.0%)	7 (100.0%)
Sex	% male	38 (76.0%)	6 (85.7%)
Age (14–77 years)	Mean (± SD) years	31.8 (± 12.8)	28.6 (± 6.1)
Weight (42–102 kg)	Mean (± SD) kg	67.3 (± 14.7)	78.0 (± 14.3)
State of infection (probable)	% Mato Grosso	27 (54.0%)	3 (42.9%)
Use of primaquine	% yes	34 (68.0%)	5 (71.4%)
Spleen size (0–5 cm)*	≥1 cm	19 (40.4%)	2 (33.3%)
Malaria episodes (1–50)	Mean (± SD) episodes	5.8 (± 10.0)	8.9 (± 14.1)
Days with symptoms prior baseline (1–24)	Mean (± SD) days	6.5 (± 4.7)	4.4 (± 3.0)
Parasitemia at baseline (3–966 parasites per 200 white blood cells)	Mean (± SD) parasites per 200 white blood cells	234.4 (± 229.3)	282.6 (± 303.1)
Drug administration	% supervised	41 (82.0%)	7 (100%)
Final total dose of primaquine received (2.06–5.00 mg/kg)†	Mean (± SD) mg/kg	3.26 (± 0.68)	2.76 (± 0.52)
Days for parasite clearance (1–7 days)‡	% 1–4 days	29 (70.7%)	4 (57.1%)
Vomiting during treatment	% yes	0 (0.0%)	0 (0.0%)
Diarrhea during treatment	% yes	8 (16.0%)	0 (0.0%)
Travel to endemic areas during follow-up	% yes	12 (24.0%)	0 (0.0%)

\* Spleen size was measured in a supine position as centimeters below the coastal margin in the mid-clavicular line.  
 † Final total dose of primaquine received was calculated dividing the standard dose received (210 mg) by the patient's weight at baseline.  
 ‡ Proportion of missing data ranged from 0% to 6%, except for days for parasite clearance (missing data = 18.0%).

of body weight, and only five (10.4%) patients received total doses of at least 4.2 mg/kg of body weight. All patients cleared their parasitemia within seven days after of the start of therapy, with the majority (70.7%) clearing parasites within four days. None reported vomiting within 1 hr after medication, and 16.0% of patients reported diarrhea during treatment. No important side effects were reported or observed during treatment of the primary vivax malaria episode. Twelve (24%) patients reported travel to endemic areas during follow-up.

Among the 50 patients analyzed, seven relapses (treatment failures) were observed. Relapses occurred on days 33, 73, 82, 88, 113, 121, and 137 after treatment initiation at a rate of 2.4 relapses per 100 person-months (7 relapses/289.4 person-months). Detailed characteristics of patients who relapsed are presented in Table 1. All relapsing patients were from the group in whom investigators supervised administration of all doses. None of them reported diarrhea or vomiting of study medications and none traveled to endemic ar-

eas during follow-up. There were six males and one female who relapsed (mean ± SD age = 28.6 ± 6.1 years). Splenomegaly was observed in two relapsed patients. In general, they did not differ from the overall population. However, the mean total dose of primaquine (mg/kg) was lower for the patients who relapsed (mean ± SD = 2.76 ± 0.52 mg/kg) than for the overall population of patients (mean ± SD = 3.26 ± 0.68 mg/kg) or for those who did not relapse (mean ± SD = 3.35 ± 0.67 mg/kg). The one female patient who relapsed received a total primaquine dose of 3.5 mg/kg. The remaining six patients (all males) who relapsed received total primaquine doses of 3.3, 3.0, 2.6, 2.5, 2.3, and 2.1 mg/kg, respectively. Of the relapsing patients, four patients cleared their parasitemia by day 3 and the other three patients by day 5 after treatment initiation.

All relapsed patients were re-treated and received chloroquine (3 days: total dose = 1.5 g) and double daily-doses of primaquine (30 mg/day over a 14-day period). No important side effect was reported or observed during re-treatment, except for one patient who presented with jaundice. This patient was re-treated for all subsequent relapses of malaria symptoms and parasitemia with chloroquine (3 days: total dose = 1.5 g) alone. One of the seven patients who was re-treated with a double dose of primaquine relapsed again after a few months. He was successfully treated with a double dose after a second re-treatment course. The remaining six patients did not return to the clinic with malaria symptoms after re-treatment during the study period.

A follow-up period of at least 4.6 months was necessary to observe all the relapses of this cohort. Although it is not possible to predict how many relapses occurred after study termination, based on these results it was observed that the hazard of relapses decreased substantially after five months of follow-up. Survival curves are shown for different total doses of primaquine (Figure 1). These curves show a higher relapse rate with decreasing dose.

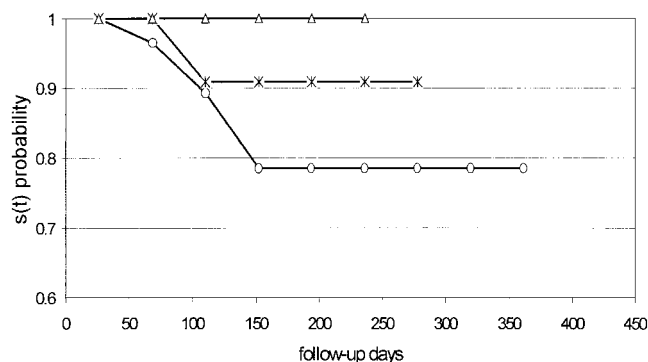


FIGURE 1. Non-relapse rates after standard vivax treatment, stratified by total dose of primaquine received (n = 48).  $\Delta$  = total dose  $\geq$  4.2 mg/kg (n = 5). \* = total dose 3.5 to > 4.2 mg/kg (n = 13).  $\circ$  = total dose < 3.5 mg/kg (n = 30).

TABLE 2

Crude and adjusted hazard ratios (HRs) from Cox regression models to assess the association between selected predictors and relapse rates during follow-up among patients treated for vivax malaria with primaquine (14 days) and chloroquine (3 days)\*

Variable	Category (or range if continuous)	Crude		Adjusted†	
		HR	P value	HR (95% CI)	P value
Age	14–77 years	0.98	0.628	–	–
Sex	0 = female, 1 = male	2.11	0.488	0.03 (0.001–0.893)	0.043
Parasitemia at baseline	3–966 parasites per 200 white blood cells	1.00	0.580	1.00 (1.000–1.008)	0.051
Days with symptoms prior to baseline	1–24 days	0.84	0.199	0.62 (0.387–1.010)	0.055
Malaria episode	1–50 episodes	1.02	0.501	–	–
Total dose of primaquine received	2.1–5.0 mg/kg	0.20	0.028	0.02 (0.001–0.291)	0.005
Days for parasite clearance	1–7 days	1.24	0.494	2.46 (0.897–6.727)	0.080
Overall chi-square (P value)				–	17.79 (P = 0.003)‡
Log likelihood (Pseudo R <sup>2</sup> )					–15.28 (R <sup>2</sup> = 0.368)

\* Proportion of missing data ranged from 0% to 22%.

† Adjusted HRs and 95% confidence intervals (95% CIs) from final Cox regression model using backwards automatic model selection (P < 0.10); n = 39.

‡ Proportional hazard (PH) assumption test for the adjusted model: P = 0.51 (no evidence of violation of the PH assumption).

To identify factors that could be statistically related to the relapse rate in the study population, associations between baseline characteristics of patients and occurrence of relapses were analyzed using Cox's proportional hazards regression. Results of crude models and adjusted final model derived by backward automatic model selection are shown in Table 2. Crude analysis identified only one variable associated with relapses: the total mg/kg primaquine dose (P = 0.028). Other variables not statistically associated (P ≥ 0.20) with relapse rate in the crude analysis were sex, age, parasitemia at baseline, days with symptoms prior to baseline, malaria episodes, and days for parasite clearance.

The full model for the backward automatic model selection procedure was based on variables shown in Table 2. It included the most biologically plausible predictors of treatment failure. This full model was limited to this set of variables due to the low power of this analysis (n = 7 relapses). The crude association between days from last primaquine dosage (19 or more days) and time to relapse was marginally not significant (P = 0.08), but this association was not analyzed in the multivariate analysis due to power limitations, because this question was poorly answered.

A final Cox model identified as risk factors for relapse (Table 2) sex (female, P = 0.043), higher parasitemia at baseline (P = 0.051), shorter number of days with symptoms prior to baseline (P = 0.055), and lower total mg/kg dose of primaquine (P = 0.005). Crude analysis of the association between sex and time to relapse identified that males had twice the probability for relapsing than females (HR<sub>(crude)</sub> = 2.11), but this association was not statistically significant. In fact, 6 males relapsed out of 6,492 male person-days (relapse rate = 0.0009), and only 1 female relapsed out of 2,189 female person-days (relapse rate = 0.0005). However, multivariate analysis showed the opposite (HR<sub>(adjusted)</sub> = 0.03, P < 0.05): females were more likely to relapse than males, independent of the total mg/kg dose of primaquine received. This happened because total dose of primaquine is a confounder for the association between sex and relapses: total dose of primaquine is a risk factor for relapses, it has a negative association (linear regression coefficient β = -0.86) with sex (female = 0, male = 1), and there are other weight-independent factors putting women at risk of failure (the female who relapsed was the only relapse observed

among patients receiving adequate mg/kg dose of primaquine).

The association between relapse rate and days for parasite clearance was marginally not significant (P = 0.080), but this variable was kept in the final model because the P value was less than the cutoff value (P < 0.10) defined for the backwards model selection. Overall, the final Cox model is highly significant (P = 0.003) and explains approximately 36.8% (pseudo R<sup>2</sup>) of the outcome (relapse rate) variability.

#### DISCUSSION

This study, which ensured compliance with medication administration and ruled out the possibility of reinfection, showed a cure rate of 86% after the standard vivax treatment in Brazil (chloroquine plus primaquine) to clear parasitemia and prevent relapses during an average of six-month follow-up period. The relapses occurred between days 33 and 137 after treatment initiation. An absence of failure during the first 28 days indicates a high cure rate of chloroquine (and perhaps primaquine) against the blood stages of *P. vivax*. Failures beyond 28 days are indicative of the failure of primaquine to kill the hypnozoites. Although serum drug levels were not obtained, there were no reports of vomiting of medications and diarrhea was minimal, indicative of adequate drug absorption. *In vivo* failures, which occur under these conditions, have been used by many as evidence of parasite resistance to primaquine.<sup>4,8,13–15</sup> However, given that the baseline efficacy of primaquine to prevent relapse varies among different geographic strains, it has been suggested that the best evidence for the development of parasite resistance would be to show a trend towards poor cure rates over time for a given geographic location.<sup>9</sup> Surely, this assumes that all other important risk factors, such as malaria immunity, patient weight, sex ratio, and drug quality are similar over time (or the analysis must adjust for them).

Our study identified some of these important risk factors: weight-dependent doses, sex, baseline parasitemia, and duration of symptoms prior to baseline. Less obvious than the others is the association between sex and relapses. This association may be due to differences in absorption, metabolism, hormonal interference, or excretion. Unfortunate-

ly, there are no similar sets of previously collected data from South America to which the cure rate of primaquine observed in this study can be compared. Perhaps this study can serve as the baseline for monitoring future trends of primaquine cure rates. If *in vitro* methods for *P. vivax* culture and drug sensitivity testing were developed, it may be possible to confirm suspected cases of parasite resistance.

Our study does not rule out the possibility that parasite resistance is occurring. If or when low-grade resistance first appears, it may be expected that it will initially cause failures in those with the highest risk factor profile for treatment failure, similar to what we have seen in this study. Moreover, although we have identified significant risk factors of failure, together they only explain a portion of the variability of treatment response (Pseudo  $R^2 = 36.8\%$ ). Even when these variables are considered, there may be other determinants not measured by our study that could also contribute to failures. The most obvious determinant would be the presence of resistant parasite strains in the study area.

This study shows that the factor most strongly associated with relapse was low mg/kg dose of primaquine. This variable should be examined before parasite resistance is considered because it may statistically account for a large proportion of observed failures. Studies have used standard, weight-independent doses of primaquine and have not examined weight, as well as other determinants of treatment failure.<sup>8,15</sup> Since the present study showed a large proportion of patients receiving a daily dose of primaquine less than the therapeutic level (3.5–4.2 mg/kg total dose), and an association with relapses, there is a need to adjust for these factors before making claims of parasite resistance.

The usefulness of these findings operationally, however, should be seen as an independent issue. It is important to consider what would be the limitations and advantages (cost-benefits) in malaria control programs if weight-adjusted doses of primaquine were recommended. Besides determining each patient's weight, there is a worry that pills would have to be split or reformulated to smaller doses so that adjustments could be made to a corrected daily mg/kg dose. Instead, if one considers only the total dose, adjustments for weight can be made by changing the total number of pills administered (either more pills on certain days of a 14-day regimen or similar daily doses for a variable number of days). On the other hand, increased operational complexity in routine procedures must be weighed against their benefits, which in turn may change if parasite resistance develops and can be shown to lead to a reduced primaquine cure rate.

In conclusion, these results indicate that the occurrence of relapses following supervised treatment of vivax malaria is in principle a necessary, but not sufficient, component of *in vivo* parasite resistance. Trend towards poor cure rates over time for a given geographic location should be considered. Moreover, the interference of other factors must be investigated, which may explain, either partially or completely, the emergence of such treatment failures. Clearly, the standard treatment assumption for vivax malaria of a patient's weight of 50–60 kg is not realistic for a large number of Brazilian male patients. Consequently, sub-dosing of primaquine occurs and may, depending on the strain of vivax parasites in

the area, contribute to a high relapse rate. Moreover, in the absence of prior primaquine resistance studies to use as baseline, it is difficult to assume that these same doses of primaquine had significantly better cure rates in previous years, or even if previous populations had similar risk factors such as weight, sex, and immunity. In this regard, for example, it is possible that previously malaria affected entire villages whereas now it is an occupational disease affecting mainly adult males. In light of what is known today, such baseline data would help to identify development of resistance in a given geographic area, and would improve the validity of conclusions from primaquine resistance studies.

**Acknowledgments:** We thank Dr. Kayla Laserson, laboratory technicians Jonilson Moraes da Silva and Laurenil Batista de Azevedo, medical students Tânia Oliveira Mendes and Cristiane Vilela Ojeda, and the staff from the Julio Muller Hospital and National Health Foundation at Mato Grosso State for their support.

**Financial support:** This work was supported by FUNASA (Health Ministry of Brazil—National Health Foundation) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico—Brazil).

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#### REFERENCES

1. Krotoski WA, 1980. Frequency of relapse and primaquine resistance in Southeast Asian vivax malaria. *N Engl J Med* 303(10): 587.
2. Peters W, 1984. Primaquine and other 8-aminoquinolines. Peters W, Richards WHG, eds. *Antimalarial Drugs I: History and Current Status of Drug Resistance*. Berlin: Springer-Verlag, 417–445.
3. Boulous M, Amato Neto V, Dutra AP, di Santi SM, Shiroma M, 1991. Frequência de recaídas de malária por *Plasmodium vivax* em uma região não-endêmica. *Rev Inst Med Trop Sao Paulo* 33: 143–146.
4. Phillips EJ, Keystone JS, Kain KC, 1996. Failure of combined chloroquine and high-dose primaquine for *Plasmodium vivax* malaria acquired in Guyana, South America. *Clin Infect Dis* 23: 1171–1173.
5. Gascon J, Gomez Arce JE, Menendez C, Valls ME, Corachan M, 1994. Poor response to primaquine in two cases of *Plasmodium vivax* malaria from Guatemala. *Trop Geogr Med* 46: 32–37.
6. Benenson AS, ed, 1995. *Control of Communicable Disease in Man*. 16th edition. Washington, DC: American Public Health Association (APHA).
7. Ministério da Saúde do Brasil, FUNASA-CENEPI, 2000. *Doenças Infecciosas e Parasitárias: Aspectos Clínicos, Vigilância*

- Epidemiológica e Medidas de Controle—Guia de Bolso*. Second edition. Brasília, Brazil: Ministério da Saúde.
8. Signorini L, Matteelli A, Castelnuovo F, Castelli F, Oladeji O, Carosi G, 1996. Short report: primaquine-tolerant *Plasmodium vivax* in an Italian traveler from Guatemala. *Am J Trop Med Hyg* 55: 472–473.
  9. Collins WE, Jeffery GM, 1996. Primaquine resistance in *Plasmodium vivax*. *Am J Trop Med Hyg* 55: 243–249.
  10. StataCorp, 1999. *Stata Statistical Software: Release 6.0*. College Station, TX: Stata Corporation.
  11. Cox DR, 1972. Regression models and lifetables. *J R Stat Soc* 34: 187–220.
  12. Grambsch PM, Therneau TM, 1994. Proportional hazards tests and diagnostic based on weighted residuals. *Biometrika* 81: 515–526.
  13. Jelinek T, Nothdurft HD, von Sonnenburg F, Loscher T, 1995. Long-term efficacy of primaquine in the treatment of vivax malaria in nonimmune travelers. *Am J Trop Med Hyg* 52: 322–324.
  14. Yi KJ, Chung MH, Kim CS, Pai SH, 1998. A relapsed case of imported tertian malaria after a standard course of hydroxychloroquine and primaquine therapy. *Korean J Parasitol* 36: 143–146.
  15. Smoak BL, DeFraités RF, Magill AJ, Kain KC, Welde BT, 1997. *Plasmodium vivax* infections in U.S. Army troops: failure of primaquine to prevent relapse in studies from Somalia. *Am J Trop Med Hyg* 56: 231–234.