

Efficacy and Effectiveness of Mefloquine and Artesunate Combination Therapy for Uncomplicated *Plasmodium falciparum* Malaria in the Peruvian Amazon

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Abstract. We evaluated the efficacy and effectiveness of mefloquine (MQ) plus artesunate (AS) to treat patients with uncomplicated malaria in the Peruvian Amazon Basin in April 2005–March 2006. Patients ≥ 1 year of age with fever (axillary temperature $\geq 37.5^\circ\text{C}$) or history of fever and *Plasmodium falciparum* mono-infection were included. Patients received antimalarial treatment with MQ (12.5 mg/kg/day for two days) and AS (4.0 mg/kg/day for three days) either by directly observed therapy or without directly observed therapy. After a 28-day follow-up, treatment efficacy and effectiveness were assessed on the basis of clinical and parasitologic outcomes. Ninety-six patients were enrolled in each study group; nine patients were lost to follow-up. All patients, except for one in the observed group, demonstrated adequate clinical and parasitologic response; none had detectable parasitemia on day 3. The efficacy of MQ + AS efficacy was 98.9% (95% confidence interval = 94.1–100.0%) and the effectiveness was 100.0% (95% confidence interval = 95.9–100.0%). Our study shows that MQ + AS is highly efficacious in the Peruvian Amazon.

INTRODUCTION

Plasmodium falciparum resistance to antimalarial drugs concerns health officials worldwide. After efficacy studies in the late 1990s and early 2000s showed treatment policies at the time were not as efficacious as desired, the Peruvian Ministry of Health decided to evaluate alternative antimalarial combinations. Efficacy studies in the Peruvian Amazon Basin showed that mefloquine (MQ) plus artesunate (AS) was highly efficacious and had a low incidence of side effects. Conversely, sulfadoxine–pyrimethamine (SP) was associated with higher levels of clinical and parasitologic failures in this region.^{1,2} This information served as the basis for the start of the implementation of MQ + AS as first-line treatment of patients with uncomplicated *Plasmodium falciparum* illness in the Peruvian Amazon region in 2001.³

Efficacy is related to how well a drug performs under ideal and controlled environments, i.e., when patients receive drugs under direct observation. Conversely, effectiveness is related to how well a drug works under real world conditions, such as when patients are instructed to take the medication on their own. Therefore, effectiveness is related not only to the properties of the drug itself to treat a disease or condition, but also to its tolerance and ease of use by patients. Poor patient adherence to recommended antimalarial regimens may contribute to treatment failures, limit the overall effectiveness of the treatment, and ultimately contribute to the development of drug resistance.⁴ The current antimalarial treatment policy in Peru is to provide treatment under directly observed therapy (DOT), but there are anecdotal reports suggesting this policy can be challenging to achieve in some areas of the country.⁵

Monitoring of antimalarial policy efficacy at regular intervals is necessary to detect the emergence of drug resistance and inform health authorities about the need for policy changes. As part of the surveillance system for resistance to antimalarial drugs, we conducted an evaluation to determine

the efficacy and effectiveness of the combination of MQ + AS for treatment of patients with uncomplicated malaria caused by *P. falciparum* in the Peruvian Amazon Basin.⁶

METHODS

Study sites. The study was conducted at the Moronacocho and Santa Clara Health Centers in Iquitos, the largest city in Loreto Province, Peruvian Amazon Basin during April 2005–March 2006. These healthcare facilities treat patients from surrounding urban and rural areas. Loreto is the province with the highest incidence of malaria in Peru (67.5% of national cases of *P. falciparum* infection in 2003), and is the only province considered to be at high risk for malaria in the country.⁷

Patients and procedures. This study followed the recommendations of the World Health Organization (WHO) with adaptations from the Pan American Health Organization for *in vivo* antimalarial drug efficacy testing in areas of low transmission.^{8–10} We included patients ≥ 1 year of age with fever (axillary temperature $\geq 37.5^\circ\text{C}$) or history of fever in the previous 48 hours. In addition, patients had to have mono-infection with *P. falciparum*, parasitemia levels between 250 and 100,000 asexual parasites/ μL , and no signs of severe malaria.

After written informed consent was provided, patients were randomly assigned to one of two study groups: observed and nonobserved therapy. Treatment consisted of AS (A.C. Farma Laboratories S.A., Lima, Peru) given at a daily dose of 4.0 mg/kg/day for three days (maximum dose of 250 mg/day) and MQ (A.C. Farma Laboratories S.A) given at a daily dose of 12.5 mg/kg/day for two days (maximum dose of 750 mg/day). Per Peruvian national guidelines, treatment with AS was started on the day of enrollment (day 0), and treatment with MQ was started the following day (day 1). Patients in the observed group received all doses under direct observation; patients in the nonobserved group received only the first dose of AS under direct observation on day 0 and were instructed to take remaining doses of AS and MQ at home by using standard written instructions. In case of vomiting within 30 minutes, the dose was repeated, and, if vomiting reoccurred within

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30 minutes after repeated dose, patients were excluded from the study and referred for treatment at the healthcare facility.

We asked patients to return to the healthcare facilities on days 1, 2, 3, 7, 14, 21, and 28 for clinical and laboratory follow-up. Axillary temperature was measured and thick blood smears were prepared at these visits. If a patient could not be located on the day of the visit, study staff attempted to locate the patient at his or her home. Patients who were not located within one day of the expected visit were considered lost to follow-up and were excluded from the study at that point. Patients in the nonobserved group were not requested to return on days 1 and 2.

Sample size was calculated on the basis of an expected rate of failure in the study population of 5% and a minimum detectable difference between study groups of 15%. The probability of falsely concluding a difference between groups (alpha error) was set at 5%; statistical power was set at 80%. Another 15% of patients were added to the study groups to account for loss to follow-up. The sample size was then determined to be 104 in each group.

Thick blood smears obtained at every visit day were stained with 10% Giemsa for 10 minutes and examined at 1,000× magnification by a trained microscopist to confirm the species and parasite density. We determined parasite density by counting the number of asexual forms and gametocytes (manual counting) against 200 leukocytes. Parasitemia per microliter was estimated assuming 6,000 leukocytes/μL. A second microscopist at the Instituto Nacional de Salud in Lima, Peru blinded to the first result re-examined the thick blood smear, and, in case of discrepancies greater than 50%, the smear was read by a third microscopist. The geometric mean of the two closest results was considered the parasitemia for each reading. At least 300 fields were examined before calling a sample negative. On day 0, we obtained additional blood for determination of hemoglobin levels.

To differentiate possible reinfection and recrudescence, we collected four drops of blood onto filter paper on day 0 to be processed in parallel with samples from the same patient if he or she had a reappearance of parasitemia on or after day 7. The genetic relatedness between isolates obtained on day 0 and days of parasitemia reappearance was determined on the basis of a neutral microsatellite profile. Eight microsatellite markers (TA1, polyα, Pfg377, PfpK2, TA 109, TA81, TA42, and 2490) located in various locations of *P. falciparum* genome were amplified as previously described.^{11,12} The finding of two isolates with identical microsatellite profile was considered as recrudescence; if isolates differed in any of the loci, those were considered as part of two independent infections, i.e., reinfection.

Outcome measures. Treatment efficacy and effectiveness were evaluated among observed and nonobserved patients,

respectively, on the basis of clinical and parasitologic outcomes and study endpoints in accordance with WHO guidelines for *in vivo* efficacy monitoring.⁹ Cases classified as early treatment failure (ETF) included 1) development of danger signs (e.g., patient's inability to drink or nurse, vomiting, seizures, altered mental status, and inability to sit or stand) or severe malaria (e.g., cerebral malaria, severe anemia [hematocrit < 15%], renal insufficiency [serum creatinine level > 3 mg/dL or clinical signs], and pulmonary edema) with parasitemia on days 1, 2, or 3; 2) day 2 parasite density > day 0; 3) positive parasitemia on day 3 and axillary temperature ≥ 37.5°C; or 4) day 3 parasite density > 25% of that on day 0. Late treatment failure included late clinical failure and late parasitologic failure. Late clinical failure was defined as development of danger signs or severe malaria with parasitemia or axillary temperature ≥ 37.5°C with parasitemia during days 3–28 without meeting criteria of ETF. Late parasitologic failure was the presence of parasitemia on days 7–28 and an axillary temperature < 37.5°C without meeting criteria of ETF or late clinical failure. Adequate clinical and parasitologic response (ACPR) included patients who did not fulfill the criteria of ETF or late treatment failure. Patients with treatment failure were treated with appropriate doses of quinine and clindamicin for seven days, according to treatment guidelines in Peru.

Statistical analysis. We entered data into a Microsoft Access (Microsoft Corp., Redmond, WA) database and used SAS version 9.1 (SAS Institute, Cary, NC) for data analysis. Dichotomous variables were compared by using the chi-square test or Fisher's exact test when expected cells counts were small. The Shapiro-Wilk test was used to assess normality of continuous variables, and the *t* test or Wilcoxon test was used to compare means of continuous variables, as appropriate. Confidence intervals around the efficacy rates for the two groups were calculated by using exact methods for a binomial proportion. A *P* value < 0.05 was considered statistically significant throughout the evaluation.

Ethical considerations. Informed consent from patients ≥ 18 years of age and from caregivers of patients < 18 years of age was obtained. In addition, we obtained assent from all participants 8–18 years of age. This study was reviewed and approved by the ethical review boards of the Instituto Nacional de Salud from Peru and the Institutional Review Board of the United States Centers for Disease Control and Prevention (CDC) (Institutional Review Board no. 4476). This evaluation was also registered under ClinicalTrials.gov (Identifier: NCT00164216).

RESULTS

A total of 192 patients were enrolled in the study, 96 in each study group. Patients' characteristics at enrollment are shown in Table 1. Randomization of patients into each study

TABLE 1
Characteristics of 192 enrolled patients by study group, Iquitos, Peru, 2005–2006

Variable	Observed group (n = 96)	Nonobserved group (n = 96)	All patients	<i>P</i>
Median age, years (range)	22.5 (3–76)	27 (3–78)	24.5 (3–78)	0.58
Male, no. (%)	64 (66.7)	60 (62.5)	124 (64.6)	0.55
Median duration of fever, days (range)	3 (1–20)	3 (1–15)	3 (1–20)	0.36
Fever on day of enrollment, no. (%)	50 (52.1)	47 (49.0)	97 (50.5)	0.67
Geometric mean asexual parasite count (parasites/μL)	3,551	5,227	4,308	0.04
Geometric mean gametocyte count (parasites/μL)	0.7684	1.1539	0.95	0.42
Mean (SD) hemoglobin level (mg/μL)	12.4 (2.2)	12.5 (2.3)	12.4 (2.2)	0.93

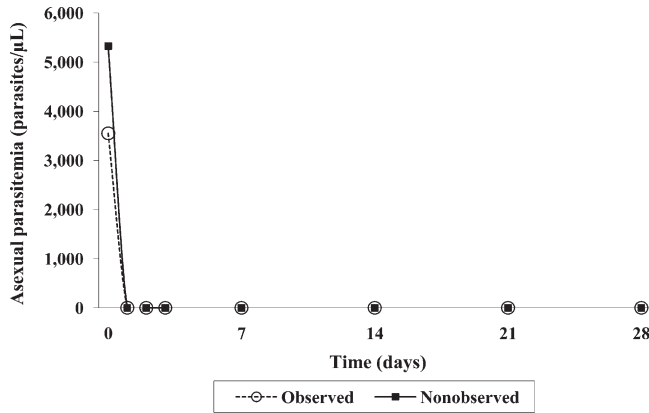


FIGURE 1. Geometric mean of asexual parasitemia of *Plasmodium falciparum* among 180 patients who completed 28-day follow up, Iquitos, Peru, 2005–2006.

groups was performed until December 2005, when the sample size for the nonobserved group was reached. Because of ethical and political concerns of increasing participants in the nonobserved arm, which is distinct from the recommendation for DOT in Peru, a decision was made to include all newly enrolled patients in the observed group from that point on until the end of enrollment. One hundred twenty-four patients were male (64.6%), the mean age was 24.5 years of age (range = 3.0–78.0 years), and 97 (50.5%) patients had documented fever at enrollment. Enrolled patients had a mean of 3.0 days of fever (range = 1.0–20.0 days) before enrollment. The geometric mean of asexual parasitemia at enrollment was 4,308 parasites/μL when all patients were considered, 3,551 parasites/μL in the observed group, and 5,227 parasites/μL in the nonobserved group; this difference was statistically significant ($P = 0.04$). There were no other statistically significant differences at enrollment between study groups for the variables analyzed.

Among the 96 patients initially enrolled in the nonobserved group, 57 (59.4%) reported having side effects after taking antimalarial treatment. Stomach ache (26 [27.1%] patients), nausea (15 [15.6%] patients), vomiting (13 [13.5%] patients),

and headache (4 [4.2%] patients) were the four most reported side effects after each dose. Despite side effects, almost all patients in the nonobserved group reported taking treatment as prescribed (93 [96.9%] patients). Three patients reported not taking pills as prescribed; two took the prescribed pills on the day subsequent to the original day and one did not take all prescribed MQ pills. None of these patients had parasitemia detected on day 3 and all were free of malaria symptoms. Forty-nine (51.0%) patients took other medicines for symptoms, such as paracetamol (26 [27.1%] patients) and dimenhydrinate (13 [13.5%] patients). On day 3, 91 (94.8%) patients stated treatment instructions received at enrollment were clear.

A total of 9 (4.7%) patients were lost to follow-up, four in the observed group and five in the nonobserved group, all of them after finishing the three-day treatment. All remaining 92 patients in the observed group completed DOT and the 28-day followup. As previously stated, among the 91 patients in the nonobserved group who finished the follow-up period, three did not complete treatment as prescribed. These patients were still followed-up for 28 days post-treatment and remained asymptomatic and had negative blood smears for malaria parasites. To avoid overestimating effectiveness by considering these patients who had ACPR despite not adhering to proposed regimen, we decided to exclude them from the outcome analysis, bringing the total number of patients in this study group to 88. The geometric means of asexual parasitemia for the 190 patients included in the outcome analysis per visit days are shown in Figure 1.

All patients demonstrated ACPR, except for one patient in the observed group. This patient was parasitemic on day 28 without fever. The parasites collected on days 0 and 28 from this patient had identical microsatellite profiles for all eight neutral markers tested, suggesting recrudescence. This recrudescence was categorized as a late parasitologic failure. Asexual parasites were not detected in any patient on day 3. In addition, fever was resolved in most patients in both groups by day 7. Fever developed in 14 patients after day 7; fevers were low grade ($\leq 37.8^{\circ}\text{C}$) in all patients, and patients were non-parasitemic on the day of fever (Figure 2). The efficacy of MQ + AS was then estimated to be 98.9% (95% confidence interval = 94.1–100.0%) in the observed group, and effectiveness

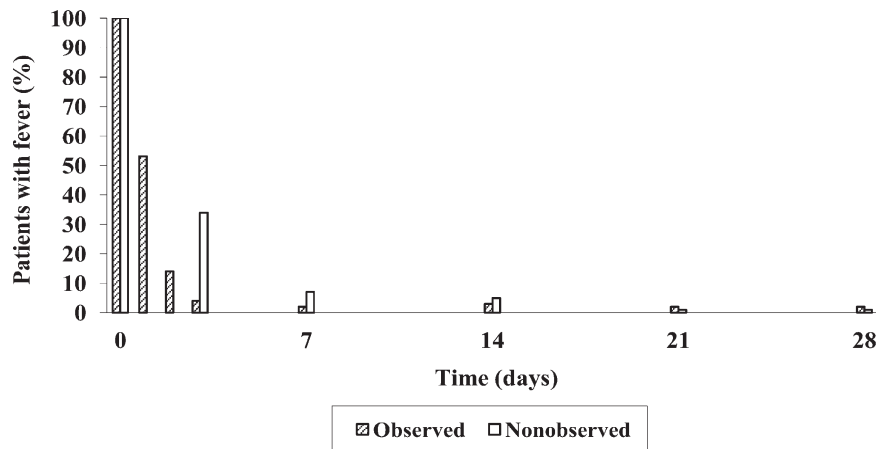


FIGURE 2. Percentage of patients with fever or history of fever since the previous visit by study group and health facility visit, Iquitos, Peru, 2005–2006.

was 100.0% (95% confidence interval = 95.9–100.0%) in the nonobserved patients, as measured by ACPR.

DISCUSSION

We estimated MQ + AS efficacy to treat patients with uncomplicated *P. falciparum* malaria to be 98.9% in the Peruvian Amazon four years after the implementation of this drug combination as first-line antimalarial treatment. Effectiveness was also evaluated and estimated to be 100%, and there was no statistically significant difference with efficacy. This evidence shows that efficacy and effectiveness are high and indicates a good treatment response to this regimen whether patients are instructed to take it by themselves or as part of a DOT strategy. In addition, all patients were negative for asexual parasites on day 3, which is indicative of high parasite's sensitivity to this regimen and artemisinin derivatives.¹³

Our results are consistent with evaluations of MQ + AS in the study region conducted in the past few years. A 28-day *in vivo* efficacy study of MQ + AS in Iquitos, Peru in 2000 included 98 patients who randomly received either MQ alone or MQ + AS.² Mefloquine as monotherapy and MQ + AS combination efficacies were estimated to be 100%. A more recent MQ + AS evaluation in the same region evaluated efficacy and pharmacokinetics of three commercial brands of MQ (Lariam[®], Mephaquin[®], and Mefloquina-AC[®] Farma) given in combination with AS against *P. falciparum* over a 56-day follow-up period in 2004–2005.¹⁴ Efficacy for all three arms was 100%. Similar to our findings, all patients had cleared parasitemia by day 3, except one patient in the Mefloquina-AC[®] Farma study, who cleared parasitemia on day 4 only.

In addition to fast parasite clearance, artemisinin-based combination therapy is recommended to slow the emergence and spread of drug resistance. This effect is achieved by the use of antimalarial drugs with different mechanisms of action and the use of a fast-acting highly effective antimalarial drug, such as AS, in combination with a long-lasting antimalarial drug, such as MQ. This combination has been associated with reduced selection of drug-resistant parasites, especially to MQ.^{15,16} Currently, WHO recommends the use of combination therapies based on artemisinin derivatives to counter the threat of drug resistance of *P. falciparum* to monotherapies and to improve compliance to treatment regimens by decreasing the total number of treatment days.

A change in national antimalarial policy is often a complex matter. In the case of Peru, this process started when healthcare workers began to notice increasing rates of treatment failures with chloroquine (CQ) in the early 1990s, increasing the possibility of drug resistance.³ In 1996, SP became the interim first-line antimalarial policy in the Peruvian Amazon Basin until more data on efficacy could be obtained. The antimalarial policy was then changed in 2001, when MQ + AS was adopted as first-line treatment of this region in Peru, although this policy was fully implemented only in 2003. Several factors were taken into account during this process and were, to some extent, responsible for the success of this move. These factors included a collaborative effort by local, national, and international partners; inclusion of district-level healthcare workers and directors throughout the process; and use of standardized protocols to obtain data on antimalarial efficacy. In summary, the new policy in Peru was chosen and

adopted taking into consideration not only information on antimalarial efficacy, but also political and other influential factors.

Compared with other studies that evaluated MQ + AS in Peru, side effects were more commonly reported in our evaluation.^{2,14} The most common side effects reported were similar to those reported for MQ + AS, i.e., those related to the gastrointestinal tract.^{17–19} Malaria infection and MQ use can cause neurologic and gastrointestinal symptoms. Thus, the precise role of MQ in producing the effects observed is unclear. However, in our study, side effects dissuaded only three patients from completing treatment as prescribed. Even in patients who delayed the completion of treatment, there was a sharp decrease in parasitemia and improvement in clinical symptoms.

Although Peru recommends malaria treatment to be provided as DOT, in this evaluation, by having a nonobserved group, we aimed to determine if MQ + AS would have high effectiveness when patients receive only the first dose as DOT and are instructed to complete treatment on their own. Our findings are of special interest in the Amazon region, where the population is widely dispersed and access to the health-care facilities by patients is exacerbated by distance and geography.^{20,21} In addition, weekend staffing of healthcare facilities and emergency care is low, making it difficult for patients to receive DOT after hours and on weekends. Our findings demonstrate that MQ + AS can be highly effective even when not given as DOT. Patients and their caretakers reported high adherence to the drug regimen, and fever and parasitemia clearance was comparable in both study arms.

Using samples obtained during this evaluation, we conducted a secondary investigation to evaluate molecular markers for parasite drug resistance to SP for which results have been published.²² Molecular analysis demonstrated that the frequency of SP-resistant alleles, the first-line treatment of *P. falciparum* in the Peruvian Amazon until 2001, had decreased four years after the change in policy to MQ + AS. This molecular epidemiology evaluation showed how removal of selective drug pressure through antimalarial policy change affected the prevalence of drug-resistant parasites. Similarly, molecular surveillance demonstrated a decrease in the prevalence of the gene responsible for CQ resistance among *P. falciparum* isolates in Malawi after it was removed from the national guidelines.²³ A subsequent *in vivo* efficacy evaluation demonstrated CQ to be highly efficacious in this country in Africa.²⁴ Unfortunately, to date, there is no reliable molecular marker for AS resistance, and *in vivo* efficacy monitoring continues to be the gold-standard method for monitoring the efficacy of this drug. Parasite clearance on day 3 has been proposed as an early indicator of *in vivo* efficacy of AS.¹³

Molecular epidemiology, provided adequate molecular markers are available, can document the genetic basis for antimalarial resistance and be of use for drug resistance monitoring when logistics for *in vivo* studies are prohibitive. With the ever-decreasing incidence of malaria in the Amazon region, the recommendation from WHO to evaluate *in vivo* efficacy every three years has become more difficult to follow.¹⁰ For example, data collection for this evaluation took almost one year to reach adequate sample size. Molecular epidemiology can serve as a tool to evaluate the prevalence of drug-resistant isolates and be part of an early detection system for antimalarial resistance. However, it has its limitations: not all drugs

have already identified molecular markers to track resistance, and there is still uncertainty in the exact correlation of resistant genes and *in vivo* efficacy.²⁵ For this reason, *in vivo* studies using WHO guidelines continue to be the most useful alternative for guiding antimalarial treatment policies.²⁶

There are several limitations in our evaluation. The evaluation might have been too short in duration to detect late recrudescences because of the long half-life of MQ, which might have caused an overestimation of adequate response rates. Patients in the nonobserved arm were informed about the study at the time of consent, which might have affected their adherence to treatment and our estimate of effectiveness.²⁷ The high treatment adherence to treatment and consequent high effectiveness observed in our study might have been influenced because this region is a setting of low malaria transmission. In such areas, patients with malaria illness seek care and are more prone to take treatment as recommended. We used an adaptation of the WHO *in vivo* efficacy trials to assess effectiveness in a group of patients who were not supervised while taking their treatment. Some authors may consider other alternatives to measure effectiveness as more appropriate, especially in areas of high transmission.²⁸ Finally, although we have interpreted the single reappearance of parasitemia observed in our study as recrudescence, one needs to be cautious about such interpretations because some recent studies suggest a high level of genetic similarity between parasite isolates obtained in the Peruvian Amazon region.²⁹ Although this interpretation did not change substantially the final outcome of this study because of the high efficacy of this drug, it will become relevant when the efficacy of the drugs decreases. Therefore, further studies are required to develop better molecular tools that can discriminate parasite isolates in this region with a high resolution to differentiate true recrudescence from reinfection.

In summary, we provide evidence that the current drug policy (MQ + AS) for treatment of patients with malaria in Peru for the Amazon region continues to be highly efficacious and effective. Despite these findings, health authorities in Peru should continue to monitor antimalarial efficacy at regular intervals. Per WHO/Pan American Health Organization recommendations, routine surveillance should be based on conducting *in vivo* efficacy trials every three years.^{10,26} Molecular epidemiology and parasite clearance on day 3 should be collected as complementary information to assess appropriateness of current policies based on artemisinin derivatives.¹³ Finally, the investigation and evaluation of adherence, effectiveness, and their impact on the development of drug resistance are critical to the decision-making process in malaria-endemic countries.

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REFERENCES

- Magill AJ, Zegarra J, Garcia C, Marquino W, Ruebush TK II, 2004. Efficacy of sulfadoxine-pyrimethamine and mefloquine for the treatment of uncomplicated *Plasmodium falciparum* malaria in the Amazon basin of Peru. *Rev Soc Bras Med Trop* 37: 279–281.
- Marquino W, Huilca M, Calampa C, Falconi E, Cabezas C, Naupay R, Ruebush TK II, 2003. Efficacy of mefloquine and a mefloquine-artesunate combination therapy for the treatment of uncomplicated *Plasmodium falciparum* malaria in the Amazon Basin of Peru. *Am J Trop Med Hyg* 68: 608–612.
- Williams HA, Vincent-Mark A, Herrera Y, Chang OJ, 2009. A retrospective analysis of the change in anti-malarial treatment policy: Peru. *Malar J* 8: 85.
- Gbotosho GO, Happi CT, Ganiyu A, Ogundahunsi OA, Sowunmi A, Oduola AM, 2009. Potential contribution of prescription practices to the emergence and spread of chloroquine resistance in south-west Nigeria: caution in the use of artemisinin combination therapy. *Malar J* 8: 313.
- Durand SMW, Ruebush TK, Cairo J, Carrion R, Quintana F, Bellina C, Rodriguez H, Utz GC, 2004. *Assessment of the Implementation of Mefloquine-Artesunate and Sulfadoxine-Pyrimethamine-Artesunate Combination Therapies for Plasmodium falciparum Malaria in Peru*. American Society of Tropical Medicine and Hygiene Annual Meeting, Miami, Florida.
2001. *Sistema de Vigilancia de la Resistencia a Medicamentos Antimalaricos en el Peru*. Lima, Peru: Instituto Nacional de Salud.
2003. *Boletín Epidemiológico. Semana Epidemiológica No. 51*. Lima, Peru: Instituto Nacional de Salud.
- Pan American Health Organization, 1998. *Evaluación de la Eficacia Terapéutica de Los Medicamentos para el Tratamiento del Paludismo por Plasmodium falciparum sin Complicaciones en las Américas*. Washington, DC: Pan American Health Organization.
- World Health Organization, 2003. *Assessment and Monitoring of Antimalarial Drug Efficacy for the Treatment of Uncomplicated falciparum Malaria*. Geneva: World Health Organization.
- World Health Organization, 2009. *Methods for Surveillance of Antimalarial Drug Efficacy*. Geneva: World Health Organization.
- McCollum AM, Mueller K, Villegas L, Udhayakumar V, Escalante AA, 2007. Common origin and fixation of *Plasmodium falciparum dhfr* and *dhps* mutations associated with sulfadoxine-pyrimethamine resistance in a low-transmission area in South America. *Antimicrob Agents Chemother* 51: 2085–2091.
- Anderson TJ, Su XZ, Bockarie M, Lagor M, Day KP, 1999. Twelve microsatellite markers for characterization of *Plasmodium falciparum* from finger-prick blood samples. *Parasitology* 119: 113–125.
- Stepniewska K, Ashley E, Lee SJ, Anstey N, Barnes KI, Binh TQ, D'Alessandro U, Day NP, de Vries PJ, Dorsey G, Guthmann JP, Mayxay M, Newton PN, Olliaro P, Osorio L, Price RN, Rowland M, Smithuis F, Taylor WR, Nosten F, White NJ, 2010. *In vivo*

- parasitological measures of artemisinin susceptibility. *J Infect Dis* 201: 570–579.
14. Gutman J, Green M, Durand S, Rojas OV, Ganguly B, Quezada WM, Utz GC, Slutsker L, Ruebush TK II, Bacon DJ, 2009. Mefloquine pharmacokinetics and mefloquine-artesunate effectiveness in Peruvian patients with uncomplicated *Plasmodium falciparum* malaria. *Malar J* 8: 58.
 15. Nosten F, van Vugt M, Price R, Luxemburger C, Thway KL, Brockman A, McGready R, ter Kuile F, Looareesuwan S, White NJ, 2000. Effects of artesunate-mefloquine combination on incidence of *Plasmodium falciparum* malaria and mefloquine resistance in western Thailand: a prospective study. *Lancet* 356: 297–302.
 16. World Health Organization, 2010. *Guidelines for the Treatment of Malaria*. Geneva: World Health Organization.
 17. ter Kuile FO, Nosten F, Luxemburger C, Kyle D, Teja-Isavatharm P, Phaipun L, Price R, Chongsuphajaisiddhi T, White NJ, 1995. Mefloquine treatment of acute falciparum malaria: a prospective study of non-serious adverse effects in 3,673 patients. *Bull World Health Organ* 73: 631–642.
 18. Price R, van Vugt M, Phaipun L, Luxemburger C, Simpson J, McGready R, ter Kuile F, Kham A, Chongsuphajaisiddhi T, White NJ, Nosten F, 1999. Adverse effects in patients with acute falciparum malaria treated with artemisinin derivatives. *Am J Trop Med Hyg* 60: 547–555.
 19. Agomo PU, Meremikwu MM, Watila IM, Omalu IJ, Odey FA, Oguche S, Ezeiru VI, Aina OO, 2008. Efficacy, safety and tolerability of artesunate-mefloquine in the treatment of uncomplicated *Plasmodium falciparum* malaria in four geographic zones of Nigeria. *Malar J* 7: 172.
 20. Fraser B, 2006. Providing medical care in the Peruvian Amazon. *Lancet* 368: 1408–1409.
 21. Quick RE, Vargas R, Moreno D, Mujica O, Beingolea L, Palacios AM, Seminario L, Tauxe RV, 1993. Epidemic cholera in the Amazon: the challenge of preventing death. *Am J Trop Med Hyg* 48: 597–602.
 22. Zhou Z, Griffing SM, de Oliveira AM, McCollum AM, Quezada WM, Arrospe N, Escalante AA, Udhayakumar V, 2008. Decline in sulfadoxine-pyrimethamine-resistant alleles after change in drug policy in the Amazon region of Peru. *Antimicrob Agents Chemother* 52: 739–741.
 23. Kublin JG, Cortese JF, Njunju EM, Mukadam RA, Wirima JJ, Kazembe PN, Djimde AA, Kouriba B, Taylor TE, Plowe CV, 2003. Reemergence of chloroquine-sensitive *Plasmodium falciparum* malaria after cessation of chloroquine use in Malawi. *J Infect Dis* 187: 1870–1875.
 24. Laufer MK, Thesing PC, Eddington ND, Masonga R, Dzinjalama FK, Takala SL, Taylor TE, Plowe CV, 2006. Return of chloroquine antimalarial efficacy in Malawi. *N Engl J Med* 355: 1959–1966.
 25. Lim P, Alker AP, Khim N, Shah NK, Incardona S, Doung S, Yi P, Bouth DM, Bouchier C, Puijalon OM, Meshnick SR, Wongsrichanalai C, Fandeur T, Le Bras J, Ringwald P, Ariey F, 2009. *Pfmdr1* copy number and artemisinin derivatives combination therapy failure in falciparum malaria in Cambodia. *Malar J* 8: 11.
 26. Vestergaard LS, Ringwald P, 2007. Responding to the challenge of antimalarial drug resistance by routine monitoring to update national malaria treatment policies. *Am J Trop Med Hyg* 77: 153–159.
 27. Kachur SP, Khatib RA, Kaizer E, Fox SS, Abdulla SM, Bloland PB, 2004. Adherence to antimalarial combination therapy with sulfadoxine-pyrimethamine and artesunate in rural Tanzania. *Am J Trop Med Hyg* 71: 715–722.
 28. Krause G, Sauerborn R, 2000. Comprehensive community effectiveness of health care. A study of malaria treatment in children and adults in rural Burkina Faso. *Ann Trop Paediatr* 20: 273–282.
 29. Dharia NV, Plouffe D, Bopp SE, Gonzalez-Paez GE, Lucas C, Salas C, Soberon V, Bursulaya B, Kochel TJ, Bacon DJ, Winzeler EA, 2010. Genome scanning of Amazonian *Plasmodium falciparum* shows subtelomeric instability and clindamycin-resistant parasites. *Genome Res* 20: 1534–1544.