Atovaquone/Proguanil Resistance in an Imported Malaria Case in Chile

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Abstract. In November 2018, we diagnosed a cluster of falciparum malaria cases in three Chilean travelers returning from Nigeria. Two patients were treated with sequential intravenous artesunate plus oral atovaquone/proguanil (AP) and one with oral AP. The third patient, a 23-year-old man, presented with fever on day 29 after oral AP treatment and was diagnosed with recrudescent falciparum malaria. The patient was then treated with oral mefloquine, followed by clinical recovery and resolution of parasitemia. Analysis of day 0 and follow-up blood samples, collected on days 9, 29, 34, 64, and 83, revealed that parasitemia had initially decreased but then increased on day 29. Sequencing confirmed Tyr268Cys mutation in the *cytochrome b* gene, associated with atovaquone resistance, in isolates collected on days 29 and 34 and *P. falciparum* dihydrofolate reductase mutation Asn511Ile, associated with proguanil resistance in all successfully sequenced samples. Molecular characterization of imported malaria contributes to clinical management in non-endemic countries, helps ascertain the appropriateness of antimalarial treatment policies, and contributes to the reporting of drug resistance patterns from endemic regions.

Chile is one of the South American countries that are free of endemic malaria, without autochthonous cases since April 1945.¹ Although malaria has been eliminated in Chile, globalization and cultural exchange have contributed to raising awareness of the disease. Moreover, with the recent increase in imported malaria cases and the worldwide spread of drug resistance, the national health reference center in Chile (Instituto de Salud Pública [ISP]) has improved its malaria surveillance, which now includes molecular species identification from all samples and analyses of possible resistance markers in cases of clinical treatment failure.² Malaria is a disease of immediate notification in Chile as part of the Emerging Diseases Surveillance Program of the Ministry of Health.

Currently, artemisinin-based combination therapies are the first-line treatment for *Plasmodium falciparum* in most endemic regions.³ Nevertheless, in Chile, atovaquone/proguanil (AP) is used as an alternative treatment.⁴ Moreover, AP is the most widely used regimen for the prevention of malaria in travelers to endemic countries.⁵ Previous case reports and studies have shown that resistance to atovaquone is associated with point mutations of the *P. falciparum* mitochondrial cytochrome b gene (*Pf*cytb) (most commonly Tyr268Ser) and resistance to proguanil is associated with point mutations in the *P. falciparum* dihydrofolate reductase gene (*Pf*dhfr- also relevant for antifolate resistance) such as N511I, C59R, and S108N.^{6–8} A high rate of antifolate resistance is currently found in *P. falciparum* parasites across major areas of endemicity^{2,7,9}; however, the Tyr268Ser mutation in *Pf*cytb has been uncommonly reported in samples collected before therapy.^{10,11} Interestingly, a report of results from a rodent model suggested that mutations in *cytb* may not be able to naturally spread within a *Plasmodium* population because of an associated fitness cost.¹² However, these mutations can be selected by drug pressure and arise after treatment. Moreover, reported AP treatment failures tend to follow a specific pattern, in which

some parasites that are originally proguanil-resistant but atovaquone-sensitive develop de novo mutations that confer resistance during the acute infection, leading to late recrudescence after treatment.^{6,7} This does not exclude the possibility of *Pf*cytb mutations carried by parasite subpopulations, where the mutation cannot be detected because of its low frequency, or the spread of parasites with permissive genetic backgrounds that favor *Pf*cytb mutation following drug pressure. Investigation of AP treatment failures is important, especially in non-endemic countries that treat imported cases and require information about; here, we describe the first AP-resistant imported malaria case in Chile.

In November 2018, three members of an Afro-Andean musical group presented at different health centers in Santiago, Chile, with an acute febrile illness accompanied by other symptoms such as myalgia, headache, vomiting, and jaundice. The members of the group had participated for 2 weeks in a music festival in Nigeria, without taking malaria prophylaxis, and returned to Chile on October 27; each was diagnosed with falciparum malaria by thick and thin smears and rapid diagnostic tests. As a notifiable disease, the samples were sent to the ISP, where the diagnosis was confirmed by real-time PCR (qPCR).¹³ Because of the severity of their clinical presentation (renal failure), two of the patients, a 33-year-old woman and a 40-year-old man, were treated with sequential intravenous artesunate and oral AP. Both recovered after being in the intensive care unit.

The third patient, a 23-year-old man, reported the first symptoms on November 1 and attended the hospital 5 days later. He received a standard oral treatment schedule with AP (1,000 mg/400 mg once daily for 3 days), with adequate clinical response. However, on day 29 after treatment, he presented with fever, myalgia, and headache and was readmitted with clinical suspicion of falciparum recrudescence. Microscopic examination of a blood smear confirmed *P. falciparum* parasitemia. The patient was treated with oral 250 mg mefloquine tablets (initial three tablets, then two tablets after 8 hours, and then one tablet after 24 hours) and followed up for up to 83 days when an adequate clinical and parasitological response was

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FIGURE 1. Timeline of patient's history since the onset of symptoms and follow-up days after treatment. Days of sample collection during the follow-up after treatment are shown with real-time PCR results expressed in parasites/μL (par/μL). Full circles represent samples after the change of treatment to mefloquine on day 29. NA = no amplification. *Patient presented with recrudescence malaria.

observed, according to Chilean treatment guidelines.⁴ Available venous blood samples (day 0—the day of initial treatment, days 9, 29, 34, 64, and 83) from this patient were sent to the ISP for diagnosis confirmation and parasite quantification using qPCR and evaluation of molecular markers of drug resistance. PCR amplification and Sanger sequencing of the *Pf*cytb (a 650-bp fragment spanning codons 268–278), *Pf*dhfr (codons 51, 59, 108, and 164), and the *Pf*Kelch13 (propeller domain and codon positions 440–600), associated with atovaquone, proguanil, and artemisinin resistance, respectively, were performed for all the seven samples of the third patient, using previously reported methods.^{7,14}

Samples from days 0 and 9 showed a reduction in parasite density from 8,000 parasites/μL to 10 parasites/μL, respectively, according to qPCR results. However, the sample from day 29 showed an increased parasitemia of 3,400/μL, which together with the clinical manifestations confirmed a treatment failure because the patient did not travel outside Santiago after the initial malaria episode. These results are consistent with previous reports which predict that recrudescence in patients with *Pf*cytb (codon 268)-mutant parasites occurs, on average, on day 29 (95% CI: 22, 35) after treatment.¹⁵ Samples collected after mefloquine treatment showed a parasitemia reduction, with complete parasite clearance on day 83 (Figure 1). On day 64, 3 parasites/μL were reported by qPCR but not by microscopy; these results could be explained by DNA persistence after parasite clearance.

Sequencing results of the *Pf*cytb gene revealed a Tyr268-Cys mutation in samples from days 29 to 34, although results from the earlier samples (days 0 and day 9) showed the wild-type allele. The *Pf*dhfr mutation Asn511le was present in all samples before and after the treatment. We neither observed mutations at codons 59 and 108 that are commonly observed in Africa nor the rare 164L mutation. No mutations were observed in the *Pf*Kelch13 gene. The results were consistent with the clinical and parasitological disease course, specifically the reduction of the parasite load after AP treatment, representing the decline of *Pf*cytb wild-type population, but subsequent replication of the population with the Tyr268Cys polymorphism leading to parasitological and clinical recrudescence of the disease. On the other hand, day 0 and day 7 samples from the other two patients, who were adequately treated with no recrudescence, did not present any mutations in *Pf*cytb or

*Pf*Kelch13 but did harbor the *Pf*dhfr mutation Asn511le. The presence of the *Pf*dhfr Asn511le allele was not surprising, given that this allele is present in Nigeria, where these isolates were acquired.⁷ Our results suggest that *Pf*cytb mutant parasites should be suspected in a recrudescence infection after AP treatment and a change in treatment should be considered.

This report represents the first reported AP resistance in an imported case of falciparum malaria in Chile. The study highlights the usefulness of collecting accurate information on patient travel history, which is fundamental to diagnose malaria promptly and for provision of effective treatment. In addition, the molecular surveillance of imported malaria in non-endemic regions contributes to the monitoring of emerging drug resistance in endemic countries as it often reflects the situation where the parasites were acquired. The development of drug resistance poses one of the greatest threats to malaria control, and non-endemic countries need to consider this in their national treatment guidelines. Given the fact that recrudescence malaria has been reported after AP treatment, it might be worth for non-endemic countries to develop some guidelines on the recommended treatment of malaria patients returning from Africa. Consideration of alternative treatments such as Coartem or mefloquine should be considered.

This report also highlights the need to advertise and encourage the use of chemoprophylaxis in travelers visiting high-transmission malaria areas. In conclusion, a close follow-up of antimalarial treatment response is key in non-endemic countries as it allows for the detection of polymorphisms associated with drug resistance, which in turn helps ascertain the appropriateness of antimalarial treatment policies of imported cases and provides information about drug-resistant genotypes entering countries.

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