Emergence of Plasmodium falciparum triple mutant in Cambodia

We share Mallika Imwong and colleagues'¹ concern regarding the spread of single-fit multidrugresistant Plasmodium falciparum malaria parasites along the borders of Cambodia-ie, to Thailand, Laos, and Vietnam. In 2016, in Cambodia, artesunate-mefloquine (ASMQ) replaced dihydroartemisininpiperaguine (DHA-PPQ) as the firstline treatment for uncomplicated P falciparum malaria. The rationale for this combination relies on evidence that parasites carrying markers of resistance both to artemisinin (pfKelch13-580Y mutant) and piperaquine (later associated with the amplification of pfplasmepsin2)^{3,4} have regained susceptibility to mefloquine (low in vitro IC⁵⁰ [half maximal inhibitory concentration] values and the single-copy pfmdr1, the amplification of which has been associated with mefloquine resistance).² One valuable hypothesis is that these genetic events could represent counteracting resistance

mechanisms, allowing strategies of alternating mefloquine and piperaquine (or a combination of all three drugs) to be considered as reasonable treatment options.

We present a retrospective analysis of P falciparum samples collected from October, 2015, to March, 2017, in the Chey Saen District (Preah Vihear province) in northeastern Cambodia, where Médecins Sans Frontières (MSF) has been supporting the local health authorities since 2014. In October, 2015, MSF strengthened the malaria passive case detection activity by adding the systematic collection of blood samples for subsequent real-time PCR analysis.5 Over this period, the first-line treatment changed from DHA-PPQ (before February, 2016) to ASMQ (after February, 2016), and 194 clinical cases of P falciparum were confirmed (130 before and 64 after February, 2016). Pfkelch13 was successfully sequenced in 141 of 191 available samples, and the 580Y mutation was identified in 112 samples (79%); no other mutation was detected. Pfmdr1 and pfplasmepsin2 copy number were successfully analysed in 129 samples. Of 118 samples with a complete genotype, 16 displayed the *pfmdr1*



Figure: Temporal increase in the proportion of Plasmodium falciparum triple mutants K13-WT=wild-type (3D7 type) pfKelch13 allele. K13-580Y=580Y mutant pfKelch13 allele. pfmdr1=P falciparum multidrug resistant1 gene. pfpm2=P falciparum plasmepsin2 gene. *Proportion of triple mutant significantly increased between the treatment periods (6 [7%] of 85 vs 10 [30%] of 33, p=0.002, Fisher's exact test). *Proportion of double mutant significantly decreased between the treatment periods (49 [58%] of 85 vs 11 [33%] of 33, p=0.02, Fisher's exact test). and pfplasmepsin2 amplification $(\geq 1.5 \text{ copies})$, and the pfKelch13-580Y mutation, suggesting the emergence of triple-mutant P falciparum strains. Worryingly, a significant increase in the proportion of triple mutants between the treatment periods was recorded (figure). Double amplification of pfmdr1 and pfplasmepsin2 was exclusively observed among pfKelch13-580Y mutants, hinting at the successful fitting adaptation of falciparum strains in their evolutionary attempt to acquire concomitant mutations related to resistance to two different antimalarial partner drugs. However, PCR-based analysis of samples collected 28 days and 63 days after ASMQ treatment did not show parasitological treatment failure. In view of these data, vigilant drug resistance surveillance and investment in alternative treatment options are warranted.

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