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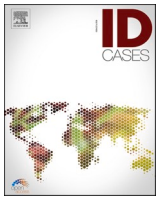

Case report

Late dihydroartemisinin-piperaquine treatment failure of *P. falciparum* malaria attack related to insufficient dosing in an obese patient

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ABSTRACT

We report the case of an obese patient who experienced late failure on day28 of a well-conducted treatment with artesunate, followed by dihydroartemisinin-piperaquine (DHA-PPQ) for a severe *P. falciparum* malaria attack. The same *P. falciparum* strain was evidenced at day0 and day28. Genotypic and phenotypic resistance tests could not explain this treatment failure. The low plasma piperaquine concentration at failure may explain the poor elimination of residual parasites.

Clinical case

We report the case of a 32-year-old man, weighing 114 kg, native from the Democratic Republic of the Congo (DRC), living in France since the age of 3, treated for a severe malaria attack after travelling in DRC in January 2019. He uses to travel regularly through sub-Saharan African capitals (DRC for 10 days by November 2018, South African and Rwanda from mid-December 2018 to mid-January 2019, then DRC up January 31 2019). He reported to have used only part of the recommended personal protection against mosquitoes (wearing appropriate clothing, using cutaneous repellent, but not of bednet) and an irregular observance of an undefined malaria chemoprophylaxis during his stay in Kinshasa. Thirteen day after returning to Paris, France, he presented to the Emergency Room of our University Hospital with a flu-like syndrome that started three days before. *P. falciparum* malaria attack was confirmed by laboratory tests, with an 11% parasitemia and no other clinical or laboratory findings of severity. He promptly received four tablets of 40 mg-dihydroartemisinin/ 320 mg-piperaquine (DHA-PPQ) (Eurartesim®), then intravenous 2.4 mg/kg artesunate administered in the medical ward. Due to favorable outcome after three doses of artesunate (at 0, 12, and 24 h), treatment was switched to Eurartesim®, four tablets per day for three days, prior to any food or drink intake [1]. On the third day following this last regimen start (day3), the patient was afebrile and thick film was negative. On day5, he was discharged home. His physical examination and complete blood counts on day7, day14, and day21 were normal.

On day28, he was readmitted with fever, headache and vomiting since 48 h. His vital signs and physical examination were normal. Laboratory tests showed thrombocytopenia (85 G/L), aregenerative anemia (Hb 11.8 g/L); plasma total bilirubin, blood lactates, and serum creatinine were in the normal range. *P. falciparum* malaria attack was diagnosed based on a positive thin blood film, showing 12% parasitemia. He presented no other signs of malaria severity and was admitted to the intensive care unit where artesunate treatment was started. His outcome was favorable after three doses of 270 mg artesunate, followed by 20 mg-artemether/120 mg-lumefantrine (Riamet®), six doses of four tablets/day. Thick films were negative on day3 and day28.

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Table 1
Genotyping of markers associated with antimalarial resistance for patient Day0 and Day28 samples.

Molecular markers	**Day0**	**Day28**
*PfCRT**		
alleles	76 T	76 T
	H97	H97
	C101	C101
	M343	M343
	C350	C350
	G353	G353
	I356T	I356T
PfCYTb		
allele	Y89	Y89
PfK13		
alleles	P441	P441
	F446	F446
	G449	G449
	N458	N458
	M476	M476
	Y493	Y493
	R539	R539
	I543	I543
	P553	P553
	R561	R561
	V568	V568
	P574	P574
	C580	C580
	A675	A675
PfMDR1		
alleles	N86	N86
	184 F	184 F
	D1246	D1246
Copy number	1	1
Plasmepsin-2		
Copy number	1	1

Day0: Day of the first malaria attack and of the first intake of artesunate
Day28: Twenty-eight day after initiating antimalarial treatment for the first malaria attack and day of relapse
PfCRT: *Plasmodium falciparum* chloroquine resistance transporter
PfCYTb: *Plasmodium falciparum* cytochrome b
PfK13: *Plasmodium falciparum* Kelch13 gene
PfMDR1: *Plasmodium falciparum* multi-drug resistance 1
*PfCRT F145I: no result

Results and Discussion

To understand the day28 failure of the classical initial treatment of this severe *P. falciparum* malaria attack, we first ruled out the following main hypotheses: patient identity theft, reinfection related to a new travel to malaria endemic area after discharge, and lack of adherence to the initial prescribed treatment (as all drug intakes were supervised by the nursing team).

Thereafter, we focused on parasitic factors that could be responsible for the treatment failure.

First, we looked for artemisinin derivatives resistance of the *P.falciparum* strain. Such resistance reported in South-East Asia is related to polymorphism of the *Pfk13* gene. In their study in Cambodia, Spring et al. reported mutations (Y493H, R539T, I543T, and C580Y) associated with decreased parasite clearance [2,3]. The most informative marker of piperaquine resistance is *plasmepsin 2* (*Pfpm2*) copy number because this increased number of copies of the *Pfpm2* gene associated with the *Pfk13* C580Y mutation and a single copy of the *mdr1* gene may contribute to failure of DHA-PPQ [4].

In sub-Saharan Africa, effectiveness of artemisinin derivatives seems to be preserved, despite their widespread use. Several studies in Africa, notably in Cameroon [5], South Africa [6], and Ghana [7] established that *Pfk13* gene polymorphism did not result in therapeutic consequences. Non-synonymous mutations *Pfk13* are rare in Africa, however a recent study [8] has highlighted the emergence of a clone carrying the mutation *Pfk13* R561H conferring resistance to artemisinin in Rwanda, near to the border with DRC. A second report confirmed the emergence of non-synonymous mutations *Pfk13* in Rwanda [9] and the presence of the mutation *Pfk13* R561H, which is alarming because it could indicate a growing resistance against antimalarials commonly used in this region of Rwanda.

Table 2
50% inhibitory concentration (IC50).

IC50	Day28	Resistance if
Dihydroartemisinin	0.56 nM	> 12 nM
Piperaquine	7.15 nM	> 90 nM
Lumefantrine	5.10 nM	> 150 nM

To study molecular markers, total genomic DNA was extracted from total blood sampled from our patient at the initial diagnosis (day0) and relapse (day28), using the MagNA Pure LC DNA Isolation kit (Roche) according to the manufacturer's recommendations. The molecular markers reported to be associated with both artemisinin derivatives resistance (*Pfk13* gene) and antimalarial partner drugs in ACT as piperaquine or lumefantrine (single nucleotide polymorphisms in *Pfcrt* and *Pfmdr1* genes, and number of copies of the *Pfmdr1*, *Pfpm2*, and *Pftrx1* genes) were sought in the two parasite isolates [10–12]: the profiles of the two isolates were identical. In both day0 and day28 isolates, we found *Pfcrt* 76 T mutation associated with chloroquine resistance. Others *Pfcrt* mutations (H97Y, C101F, C350R, M343L and G353V) reported to be associated with PPQ resistance have been explored and all were wild in both day0 and day28 isolates [13]. Both no mutation *Pfcyt b* gene mutation associated with atovaquone resistance and no *Pfk13* gene mutation associated with artemisinin derivatives resistance were found. A N86/184 F/D1246 profile in the *Pfmdr1* gene known to be associated a decreased lumefantrine (but not piperaquine) sensitivity [10], and a copy number equal to 1 for both *Pfmdr1* and *Pfpm2* genes, supporting the absence of resistance to lumefantrine (PfMDR1) or piperaquine (PM2) [11] have been observed (Table 1).

To definitively rule out a new infection by another *P. falciparum* isolate in our patient and to evaluate the clonality of the day0 and the day28 isolates, 5 microsatellites (TAA81, TAA87, TAA60, ARA2, and PFPK2) on different chromosomes were genotyped, according to the method described by Musset et al. [12]. The two isolates presented the same clone $(TAA81)_{187}$, $(TAA87)_{96}$, $(TAA60)_{88}$, $(ARA2)_{103}$, $(PfPK2)_{187}$.

These results show that the two malaria attacks described here were due the same *P.falciparum* strain, without any indication of genotypic resistance to artemisinin derivatives or to the antimalarial partner drug, and thus confirm the hypothesis of recrudescence of the initial parasite isolate.

It was not possible to study the *ex vivo* sensitivity to artemisinin derivatives of the day0 *P. falciparum* isolate, but the day28 isolate showed dihydroartemisinin, piperaquine, lumefantrine 50%inhibitory concentrations (IC_{50}) of 0.56 nM, 7.15 nM, and 5.10 nM, respectively, that is no decreased sensitivity for any of these drugs [14,15] (Table 2). Unfortunately, it was not possible to assess the *in vitro* artemisinin sensitivity by the RSA test [2]. However, the absence of parasitaemia three days after the start of the treatment (for both first attack and the relapse) did not support resistance to the artemisinin derivative defined by delayed parasites clearance.

We then investigated the hypothesis of a too low DHA-PPQ dosing, given the patient's high body mass index (30.9 kg/m^2). The high distribution of piperaquine in adipose tissue could explain the lower plasma piperaquine concentrations observed in obese patients [7]. A case of DHA-PPQ treatment failure was reported in a patient weighing 104 kg [16] and the authors hypothesized that this was due to insufficient antimalarial dosing, although not supported by plasma concentration measurement.

The DHA-PPQ dosing recommended for the treatment of malaria attacks depends on the patient's bodyweight [17], but there are no data or recommendations if exceeding 100 kg [18]. Our patient received a

Table 3
Plasma piperaquine concentration.

Piperaquine (ng/mL)	Day7	Day28
Assay values	106	6.5
Expected values	> 30	> 10

Day7: Seventh day after initiating antimalarial treatment for the first malaria attack
Day 28: Twenty-eighth day after initiating antimalarial treatment of the first malaria attack (day of relapse)

total dose of piperaquine of 3840 mg over three days, that is an average of 33 mg/kg. The recommended dose in children to avoid recrudescence is between 48 mg/kg and 59 mg/kg. The risk of recrudescence is increased by 13% with every 5 mg/kg dose decrease [19].

We assayed plasma piperaquine concentration using high-performance liquid chromatography coupled to tandem mass spectrometry in samples collected on day7 after diagnosis of the first malaria attack (day4 post-DHA-PPQ first dose), and at diagnosis of recrudescence (day25 post-DHA-PPQ first dose) (Table 3). The plasma piperaquine concentration 7 day after the start of DHA-PPQ treatment is reported to be predictive of the risk of recrudescence and a value below 30 ng/mL is reported to be associated with the risk of treatment failure [20]. However, since the patient's samples were not collected specifically for pharmacological monitoring of piperaquine, we were unable to draw definitive conclusions. While the piperaquine plasma concentration on day7, that is on day4 post-DHA-PPQ treatment, was within the expected range, it was low on day28 (that is on day25 post-DHA-PPQ treatment) as compared to expected values of at least 10 ng/mL reported in the meta-analysis of Hoglund et al. [21]. This finding suggests an insufficient piperaquine dosing to clear residual parasites after the action of artemisinin derivatives, which constitutes the rationale of artemisinin-based combination therapy [22].

Conclusion

To the best of our knowledge, we report here the second case of late recrudescence of *P. falciparum* malaria attack associated with low DHA-PPQ dosing, probably related to the patient obesity. The choice of oral route for treatment in malaria attacks should take into account the patient's weight and, when exceeding 100 kg, an alternative IV antimalarial treatment should be used. Pharmacokinetics and pharmacodynamics data regarding piperaquine among this population would be useful.

CRediT authorship contribution statement

Conceptualization: M.Parisey, S.Houze, S.Lariven, S.Matheron. Methodology: S.Houze. Investigation: S.Houze, N.Argy, J.Bailly, N. Taudon. Formal analysis: S.Houze, N.Argy, J.Bailly, N.Taudon. Writing - Original Draft: M.Parisey, S.Houze, N.Argy. Writing - Review & Editing: J.Bailly, N.Taudon, K.Jaffal, B.Megarbane, C.Rouzaud, Y.Yazdanpanah, S.Martheron. Supervision: S.houze, S.Matheron.

Conflict of interest

None of the authors have any conflict of interest in relation to this publication.

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