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Case Report

Artesunate and dihydroartemisinin-piperaquine treatment failure in a severe *Plasmodium falciparum* malaria case imported from Republic of Côte d'Ivoire

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ABSTRACT

A 68-year-old man returning from Republic of Côte d'Ivoire (Ivory Coast) was diagnosed with severe *Plasmodium falciparum* malaria and treated with intravenous artesunate followed by oral dihydroartemisinin-piperaquine (DHA-PPQ). A month later the patient experienced a new *P. falciparum* episode; analysis of *pfmsp-1* and *pfmsp-2* revealed that the infection was caused by a genetic strain identical to the strain that caused the initial episode, indicating resurgence of the previous infection. No mutations in genes associated with resistance to artemisinin derivatives (*pfk13*) or piperaquine (*pfexonuclease, pfplasmepsin 2/3*) were detected, suggesting that treatment failure could have been caused by drug malabsorption or poor drug manufacturing practices. A second treatment with atovaquone-proguanil was successful in eliminating the infection, with no further relapses. To our knowledge, this is the first description of a treatment failure with both artesunate and DHA-PPQ in a traveler returning from a malaria-endemic region. Analysis of molecular markers of resistance to antimalarial drugs revealed mutations associated with resistance to sulfadoxine (*pfdhps*) and pyrimethamine *(pfdhfr)*, highlighting the important contribution of surveillance of imported malaria cases to the monitoring of drug resistance globally.

Background

Malaria, an infectious disease imposing a heavy burden on human health and a major cause of illness and death at global level, is caused by protozoan parasites of the genus *Plasmodium* and transmitted by female mosquitoes of the genus *Anopheles*. In 2020, the World Health Organization (WHO) reported 241 million clinical cases and 627,000 deaths in 87 malaria-endemic countries in subtropical and tropical regions of the world, most of which (>95%) occurred in children <5 years old and pregnant women in Sub-Saharan Africa and were caused by *P. falciparum* (WHO, 2021). One of the greatest challenges in successfully controlling and eliminating malaria is the development of antimalarial drug resistance in *P. falciparum* (Dhorda et al., 2021). In Europe, malaria is a highly relevant imported disease because of travel to endemic areas. In 2015-2019, 3958 malaria cases were reported in Italy, the great majority of which were caused by *P. falciparum* (84%) and contracted in Sub-Saharan African countries (92%) (Boccolini et al., 2021). The case reported herein was observed in Pisa University Hospital, Pisa, Italy.

Case Presentation

A 68-year-old Italian man was admitted in October 2020 to the Emergency Unit (Pisa), for fever (up to 39°C) with chills. Symptoms appeared four days after his return from Abidjan, Ivory Coast, where he stayed for three weeks with no uptake of antimalarial prophylaxis. Given the clinical parameters (Table S1) and travel history, a malarial infection was suspected, and it was

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confirmed by a loop mediated amplification (LAMP) detecting *Plasmodium spp*. DNA and by optical microscopy detecting *P. falciparum* trophozoites with a parasite density of 0.8% infected red blood cells (iRBC). The patient was diagnosed with severe *P. falciparum* malaria according to WHO guidelines, because he was prostate, and was treated with intravenous artesunate (Falcigo, 60 mg/vial; three-doses, each dose consisting of 2.4 mg/kg which amounted to 180 mg,i.e, three vials, administered at diagnosis and at 12 hours and 24 hours after the first dose), and then with oral DHA-PPQ combination (Eurartesim, 40 mg/320 mg/tablet, three tablets/day for three days, which was in accordance with the Italian registration file [www.agenziafarmaco.gov.it], along with the European Medicines Agency [EMA authorization [www.ema.europa.eu] and the Sanford Guide to Antimicrobial Therapy [www.sanfordguide.com], which state that for a patient whose body weight is < 75 kg [our patient weighed 63 kg], tablets are administered starting from 24 hours after the last dose of artesunate; these tablets were taken at the same time each day with water and without food, no less than 3 hours after the last food intake and with no food taken within 3 hours after each dose; no dose was vomited or missed). Malaria microscopy showed a negative result on the seventh day from treatment after a progressive reduction of parasite density (Table S2, Figure S5). The patient was discharged after resolution of all clinical signs and symptoms. More than three weeks later, the patient was re-admitted to the same hospital with symptoms of acute malaria. LAMP was positive for *Plasmodium* spp. DNA and optical microscopy identified *P. falciparum* trophozoites with a parasite density of 0.4% iRBC. The patient was treated with atovaquone-proguanil (Malarone; 250 mg/100 mg, four tablets/day for three days) and later discharged. At follow-up visits, 7, 15, and 60 days after the second malaria episode, blood films showed a negative result. Molecular analyses were retrospectively performed to investigate reasons for malaria recurrence, in absence of further travel to endemic areas.

Materials and Methods

EDTA whole blood samples were collected from the patient at different time points during both malaria episodes (Table S2) and stored at −20°C. Total DNA was extracted using a DNA Blood Mini Kit (Qiagen, Hilden, Germany) from 200 µl of the collected whole blood sample according to the manufacturer's instructions. Species identification was performed by a nested PCR and agarose gel electrophoresis protocol described by Snounou and colleagues (1993) on samples collected before treatment. Species were also determined by a commercial real-time PCR assay (RealStar Malaria PCR kit, Altona Diagnostics, Hamburg, Germany) performed on samples collected before treatment (Day 0) and at Days 1, 4, 5 and 6 after treatment; this procedure also allowed a semi-quantitative determination of parasite density over time. Genotyping of *P. falciparum* clones was performed by length polymorphism analysis of *pfmsp-1* and *pfmsp-2* loci (Soulama et al., 2009). The presence of mutations associated with antimalarial drug resistance was investigated by PCR and amplicon Sanger sequencing of *pfk13, pfcrt, pfmdr1, pfcytB, pfexonuclease, pfdhps* and *pfdhfr* loci using previously described protocols (Table 1). Resistance to piperaquine was also evaluated by analysis of *pfplasmepsin 2/3* copy number by genotyping of breakpoint mutation using PCR and agarose gel electrophoresis using a recently described method (Table 1). Genotyping of drug resistance markers was performed on samples from both clinical episodes, collected before treatment.

Results and discussion

Species identification (Figures S1 and S2) confirmed that both malaria episodes were caused by *P. falciparum* only, and excluded the possibility that the second episode could have been a relapse caused by hypnozoites of *P. vivax* and *P. ovale*. Analysis of *pfmsp-1* and *pfmsp-2* fragment length polymorphism (Figure S3), along with analysis of sequences of antimalarial resistance loci (Table 1), revealed that infection was monoclonal and caused by an identical genetic strain of *P. falciparum* in the two episodes. These results confirmed resurgence of the infection as the cause of the second episode. Parasite density was measured by microscopy performed before treatment and at different time points after treatment (Table S2). A slower reduction in parasite density over time was observed in the first episode compared with the second (Figure S5), suggesting a suboptimal efficacy of the first antimalarial treatment and supporting the use of parasite clearance time as an indicator of recurrence risk (Landre et al., 2021). Although real-time PCR cannot be used to estimate parasite clearance time because both live and dead parasite DNA is detected, it is noteworthy that the reduction in Ct observed between day 0 and day 1 after treatment was lower in the first episode than in the second episode (ΔCt first episode=-1.54; ΔCt second episode=-4.01), corroborating the evidence provided by microscopy. Analysis of molecular markers of resistance to antimalarial drugs revealed no mutations associated with resistance to artemisinin and piperaquine but detected the presence of mutations associated with resistance to sulfadoxine and pyrimethamine (Table 1; Figure S4).

Conclusions

The case reported herein describes the recurrence of clinical *P. falciparum* malaria in a patient returning to Italy from Ivory Coast. Recurrence was due to the resurgence of infection after treatment with intravenous artesunate and oral DHA-PPQ, and molecular markers of resistance to the employed antimalarial drugs were absent. An instance of artesunate treatment failure in an imported case of severe malaria in France wasprevisously described (Landre et al., 2021), and it was not attributed to drug resistance. Two instances of DHA-PPQ treatment failure in imported cases of severe malaria in Italy were also previously described (Gobbi et al., 2016; Russo et al., 2018) and were not attributed to drug resistance, although fewer molecular markers of resistance to piperaquine were investigated than those in the present report. The current case, however, is to our knowledge the first in which recurrence of malaria was observed after treatment with both artesunate and DDH-PPQ in Europe. Because no mutations in the molecular markers of resistance to the antimalarial drugs of interest were observed, therapeutic failure may be ascribed to alternative causes such as drug malabsorption by the patient or poor drug manufacturing practices. Regarding the former, a pharmacokinetic analysis to determine the blood concentration of drugs at various time points by mass spectrometry could be useful. Regarding the latter, it is noteworthy that intravenous artesunate (IVA) products available in Europe do not comply with Good Medical Practice standards and that the product purchased by the procurement agency of the National Health System in the Tuscany region (ESTAR) is not prequalified by the WHO (https://extranet.who.int/pqweb/medicines/prequalified-lists). A request to purchase a WHO-Prequalified IVA product such as Artesun (Guilin Pharmaceutical, Shanghai, People's Republic of China), which is currently used in other European Countries, will be submitted to ESTAR following the publication of this case report. It should also be noted that the DHA-PPQ schedule recommended by WHO malaria treatment guidelines for a patient of 63-kg body weight is different from the one indicated by Italian registration file/EMA authorization/Stanford Guide to Antimicrobial Therapy (four tablets/day instead of three tablets/day). Because the patient's body weight (63 kg) was very close to the cutoff (60 kg) indicated for an increase in dosage from three to four tablets per day in the WHO malaria

Table 1
Analysis of molecular markers associated with antimalarial drug resistance.

Locus	Pfk13[a]	Pfmdr1[b]	Pfcrt[c]	Pfdhps[d]	Pfdhfr[e]	Pfcytb[f]	Pfplasmepsin 2/3[g]	Pfexonuclease[h]
Aminoacid position	[i]580	86 184 1034 1042 1246	74 75 76 218 220	436 437 540 581 613	51 59 108	258 268	-	415
Wild type	C tgt	N Y S N D aat tat agt aat gat	M N K I A atg aat aaa att gcc	S A K A A tct gct aaa gcg gcc	N C S aat tgt agc	I Y att tat	1 copy	E gag
Mutant	Y tat	Y F C D Y tat ttt tgt gat tat	I E T F S att gaa aca ttt tcc	A/F G E G S/T gct/ttt ggt gaa ggg tcc/acc	I R N att cgt aac	M S/N/C/F atg tct/aat/tgt/ttt	≥2 copies	G ggg
First episode	C tgt	N Y S N D aat tat agt aat gat	M N K I A atg aat aaa att gcc	A A K A A gct gct aaa gcg gcc	N R N aat cgt aac	I Y att tat	1 copy	E gag
Second episode	C tgt	N Y S N D aat tat agt aat gat	M N K I A atg aat aaa att gcc	A A K A A gct gct aaa gcg gcc	N R N aat cgt aac	I Y att tat	1 copy	E gag

Shown are the loci associated with resistance to antimalarial drugs investigated in the present study. For each locus, the aminoacidic position(s) of interest are indicated, together with the wild type and mutant aminoacidic and codon sequences. The results of *P. falciparum* genotyping in the first and second clinical malaria episodes are presented. Mutant genotypes are highlighted in red.

[a] Resistance to artemisinin (Taylor et al., 2015).
[b] Resistance to amodiaquine, chloroquine, lumefantrine and mefloquine (Duraisingh and Cowman, 2005).
[c] Resistance to chloroquine (Palmieri et al., 2004.
[d,e] Resistance to sulphadoxine (Menegon et al., 2009).
[f] Resistance to pyrimethamine (Korsinczky et al., 2000).
[g] Resistance to piperaquine (Ansbro et al., 2020).
[h] Resistance to piperaquine (Boonyalai et al., 2020).
[i] The position of the 10 markers of artemisinin resistance defined by the WHO were analyzed and no mutations were observed (Table S3).

treatment guidelines, we reckon that the employed dosage of DHA-PPQ did not negatively affect treatment efficacy in this case. However, a harmonization of guidelines on the basis of treatment efficacy data would be useful. Finally, although mutations associated with resistance to artemisinin and piperaquine were not observed, mutations associated with resistance to sulphadoxine and pyrimethamine were detected. This case therefore highlights the importance of surveillance of antimalarial drug resistance markers in imported malaria cases, not only to optimize the therapeutic approach but also to contribute to the global monitoring of this emerging threat to malaria control efforts (Dhorda et al., 2021, Supplementary references)

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Ethical Approval

The present work did not involve experiments on humans or animals. The privacy of the patient was protected by ensuring anonymity and confidentiality in both data management and reporting. No identifying information is contained in the case report.

Author contributions

V. Motta, S. Verdenelli contributed to data acquisition and analysis. R. Sparavelli contributed to data acquisition. S. Fabiani, V. Mangano contributed to conception and design of the study, data acquisition and analysis, drafting the manuscript. C. Severini, M. L'Episcopia, F. Bruschi contributed to conception and design of the study. All authors have revised the manuscript and approved it for pubblication.

Declarations of Competing Interest

The authors have no competing interests to declare.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2022.06.009.

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