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Case Report

Artemether-lumefantrine treatment failure of uncomplicated *Plasmodium falciparum* malaria in travellers coming from Angola and Mozambique

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ABSTRACT

The failure of artemisinin combination therapy (ACT) in malaria patients returning from endemic regions may be driven by parasite resistance to this treatment. ACT is used globally as the first-line treatment for *Plasmodium falciparum* malaria. However, artemisinin-resistant strains of *P. falciparum* have emerged and spread across Southeast Asia, with the risk of reaching high malaria burden regions in Africa and elsewhere. Here, we report on two malaria imported cases from Africa with possible parasite resistance to the ACT artemether-lumefantrine (AL).

Case presentation: Two middle-aged males returning from Angola and Mozambique developed malaria symptoms in Portugal, where they were diagnosed and received treatment with AL as hospital inpatients. After apparent cure and discharge from hospital, these individuals returned to hospital showing signs of late clinical failure. Molecular analysis was performed across a number of drug resistance associated genes. No evidence of *pfk13*-mediated artemisinin resistance was found. Both subjects had complete parasite clearance after treatment with non-ACT antimalarials.

Conclusion: Our case-studies highlights the need for close monitoring of signs of unsatisfactory antimalarial efficacy among AL treated patients and the possible implication of other genes or mutations in the parasite response to ACTs.

Background

Despite the success of artemisinin combination therapy (ACT) in reducing the global burden of malaria, the emergence and spread of resistance to artemisinin threatens its use. ACT is used worldwide as the first-line treatment against uncomplicated falciparum malaria. ACT resistance, characterized by delayed parasite clearance after treatment, is widespread in Southeast Asia (Imwong et al, 2017), but as yet there is no irrefutable evidence of an established presence in Africa (https://www.who.int/malaria/areas/drug_resistance/updates/en/). Nonetheless reports

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Table 1
Genotyping data from the two *P. falciparum* isolates taken from the recrudescence malaria episodes.

	Patient(case 1)	Patient(case 2)
Sex	male	male
Age (years)	52	56
Country of origin[a)]	Angola	Mozambique
Recrudescence time[b)]	22 days	21 days
Drug resistance associated genes	Genetic variation	
chloroquine or mefloquine		
pfcrt	CVMNK	CVMNK
pfmdr1	wt	wt
pfmdr2	wt	wt
sulphadoxine-pyrimethamine		
pfdhfr	51I, 59R, 108N	51I, 59R, 108N
pfdhps	540E	540E
artemisinin combination therapies		
pfk13	wt	wt
pfpmII	wt	wt
pfpmIII	wt	wt
pfcoronin	wt	wt
pfap2μ	199T	wt
pfubp-1	615H, 956N, 1710S, 1914N, 1915K, 2238K, 3121N	174N, 615H, 906N, 1133S, 1531D, 1660I, 1914N, 1915K, 2238K, 2818Y, 3103N

[a)] Country where patients were infected
[b)] elapsed time since primary malaria episode; wt, wild-type, sequence identical to the reference *Plasmodium falciparum* clone 3D7 (MRA-102); *pfcrt* (chloroquine resistance transporter; PF3D7_0709000.1); *pfmdr1* (multidrug resistance protein 1; PF3D7_0523000.1); *pfmdr2* (multidrug resistance protein 2; PF3D7_1447900.1); *pfdhfr* (bifunctional dihydrofolate reductase-thymidylate synthase; PF3D7_0417200); *pfdhps* (hydroxymethyl dihydropterin pyrophosphokinase-dihydropteroate synthase; *pfk13* (kelch13; PF3D7_1343700); *pfpmII* (plasmepsinII; PF3D7_1408000); *pfpmIII* (plasmepsinIII PF3D7_1408100); *pfcoronin* (coronin; PF3D7_1251200); *pfap2μ* (adaptor protein complex-2 μ subunit; PF3D7_1218300); PF3D7_0810800); *pfubp-1* (ubiquitin carboxyl-terminal hydrolase 1; PF3D7_0104300).

of therapeutic failure have emerged, including from Angola (Van et al, 2014). Artemether-lumefantrine (AL) is the most widely used ACT in sub-Saharan Africa, with some recent reports of the emergence of decreasing efficacy, supported by patients remaining positive for Plasmodium 3 days after treatment (Madamet et al, 2017; Uwimana et al, 2020). The underlying mechanisms are not understood.

Single nucleotide polymorphisms (SNPs) in the parasite gene *pfk13* are becoming established as molecular markers of slow parasite clearance rates after ACT (Imwong et al, 2017), but they have been identified rarely in Africa (Madamet et al, 2017; Taylor et al, 2015; Sutherland et al, 2017). Hence the variable ACT efficacy may be independent of *pfk13* polymorphism, with suggested links to multi-locus genotypes encompassing *pfcrt, pfmdr1, pfcoronin, pfap2μ* or *pfubp1* genes (Sutherland et al, 2017). Specifically, haplotype NFD (N86, 184F, D1246) in *pfmdr1*, the mutation S160N/T in *pfap2μ* and D1525E, E1528D and E1531D in *pfubp-1* have been involved in the development of tolerance or resistance to AL (Henrici et al, 2020; Adamu et al, 2020). Here we present a brief case report and genotyping data of parasites (Table 1) isolated from the recurrence episode of two patients returning to Portugal from Angola and Mozambique.

Malaria is a Notifiable Disease in Portugal and AL is used for the treatment of imported cases of *P. falciparum*. All parasite genotyping tests performed were formally requested by the physicians directly responsible for patient care. Patient identifiers were removed by the physicians directly responsible for the patient. Both patients signed a written informed consent for the analysis of parasite genes, under the IHMT Ethics Committee Approval n°11.18 and CHLO Ethics Committee Approval RNEC:20170700050.

Case 1

A 52-year-old male presented to Centro Hospitalar Universitário de São João Emergency Department (CHUSJ-ED), Porto, Portugal in August 2019 with malaria-compatible symptoms after travelling from Luanda, Angola. He had a high-grade fever during the four days preceding admission and developed prostration on the day of admission (Day 0;D0). Dysfunctions on D0: neurological (prostration, disorientation), cardiovascular (hypotension with hyperlactacidaemia of 3.43 mmol/L) and hepatic (hyperbilirubinemia of 3.34 mg/dL). A malaria rapid test (BinaxNOW®) and a blood smear showed 7% parasitaemia. The patient was transferred to the Intensive Care Unit (D0) and treated with intravenous artesunate (200mg) at 0h, 12h, 24h, 48h and 72h (D3) followed by a course of AL for 3 consecutive days (4 pills AL 20/120mg at 0h, 8h, 20h, 32h, 44h and 56h). The patient was discharged on D9 of in-hospital treatment. He stayed in Portugal and did not return to Angola. Eleven days after discharge (D19) he developed high-grade fever (39°C), nausea, vomiting and general malaise. Two days afterward (D21), he presented to CHUSJ-ED. The patient did not show any relevant organ dysfunction. Analysis of blood smear led to a positive result for *P. falciparum* (confirmed by polymerase chain reaction; PCR) with 8.5% parasitaemia. The patient was treated with intravenous quinine (600mg) and doxycycline (200mg) for 7 days. Parasitaemia became negative and the patient improved. The patient was followed in the outpatient clinic and remained asymptomatic until return to Angola (D28).

Case 2

A 56-year-old male presented to the emergency department of Centro Hospitalar Lisboa Ocidental (CHLO), Lisbon, Portugal in July 2019 with symptoms compatible with malaria after returning from Mozambique, Nacala, where he was residing since 2013. For the previous two days he had malaise, chills, profuse sweating, nausea and episodic vomiting. On admission (D0) to the CHLO infectious diseases ward, the patient presented: fever (38.5°C), tachycardia without hypotension, jaundice, tender abdomen, thrombocytopenia (38×10^9/L), hyperbilirubinemia (2,55 mg/dL), elevated

liver enzymes (AST 119 U/L, ALT 434 U/L, GGT 493 U/L) and high C-reactive protein (23,9 mg/dL). The blood smear presented 6% *P. falciparum* parasitaemia with peripheral schizont clusters. He was treated (D0) with a full course of oral AL (20/120mg) for three consecutive days (four AL pills at 0h, 8h, 24h, 36h, 48h and 60h). On D3 parasitaemia was negative, along with remission of symptoms. The patient was discharged five days after admission and referred to the outpatient clinic, presenting (D8) without symptoms or parasitaemia. The patient stayed in Portugal and did not travel abroad. Symptoms recurred thirteen days later (D21) and the patient was readmitted to the hospital (D23) without evidence of organ dysfunction. A blood smear revealed 3% *P. falciparum* parasitaemia and was confirmed by PCR. The patient was successfully treated with seven days of intravenous quinine (10mg/Kg of body weight every eight hours) and doxycycline (200mg/day). The patient was asymptomatic and had clearance of parasitaemia by the fourth day of treatment (D26). He was discharged from the hospital (D29) and presented without symptoms up until his last follow-up in the outpatient clinic (D35).

Discussion

P. falciparum drug resistance genes were sequenced and analysed in parasite isolates from the recurrence episode of both patients (Table 1). In line with other findings from Africa (Madamet et al, 2017; Taylor et al, 2015; Sutherland et al, 2017) no SNPs in *pfk13* were identified. *Pfmdr1* and chloroquine-sensitive *pfcrt* haplotype (CVMNK) were present in both parasite isolates, agreeing with a significant decline in the frequency observed after withdrawal of chloroquine in Africa (Ocan et al, 2019). Mozambique withdrew chloroquine in 2002 and Angola in 2004 and both countries have been using ACTs (mostly AL) since 2006. The *pfap2*μ polymorphism identified has not been previously associated with delayed parasite clearance and only one of the previously reported *pfubp-1* polymorphisms (E1531D) (Adams et a, 2018; Henrici et al, 2020) was detected. Although the role played by both genes in artemisinin action is not fully understood, the biological processes in which they are involved make them strong candidates. *Pfap2*μ is part of the complex multigenic signature of artemisinin susceptibility in Southeast Asia (Cerqueira et al, 2017) and is essential for parasite survival inside the erythrocyte (Henrici et al, 2020). *Pfubp-1* encodes a deubiquitinating enzyme part of the ubiquitin-proteasome system, which is crucial for protein recycling and stress response. An enhanced stress response, with lower levels of ubiquitination, delays cell death and confers resistance to artemisinin (Dogovski et al, 2015). Treatment failure cannot be fully attributed to parasite resistance in these two patients since: a) no SNPs in the loci previously linked to reduced susceptibility to AL (*pfk13* and *pfmdr1*) were detected; b) measurements of lumefantrine blood levels at day 7 after treatment to rule out malabsorption (Bell et al, 2009) were not performed. Nevertheless, both patients received treatment (with a meal) as hospital inpatients and signs of malabsorption were not detected. Artemisinin derivatives are rapidly metabolized (half-life $\pm$1.5h) and cleared from circulation (Hall et al, 2013), resulting in only a single full parasite life cycle being exposed to the drug, under conditions of a 3-day regimen. Since lumefantrine half-life is 1-10 days (Bretscher et al, 2020), is the current practice of 3 days of treatment sufficient to guarantee clearance of parasites?

Conclusions

These observations reveal a need for close monitoring of signs of unsatisfactory antimalarial efficacy among AL treated patients and the possible involvement of other loci in the parasite's response to AL, especially because conditions for malaria reintroduction in Portugal are still present in the country.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical Approval statement

Both patients signed a written informed consent for the analysis of parasite genes, under the IHMT Ethics Committee Approval n°11.18 and CHLO Ethics Committee Approval RNEC:20170700050.

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