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Case Report

Plasmodium malariae after successful treatment of *P. falciparum* malaria with artemether-lumefantrine

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ABSTRACT

We described a case of *Plasmodium malariae* malaria in a traveler returning from the Democratic Republic of Congo to Belgium. This occurred despite successful artemether-lumefantrine treatment for *Plasmodium falciparum* three weeks earlier and in the absence of re-exposure in an endemic area. We discuss possible explanations for this unusual observation.

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Introduction

Five Plasmodium species cause malaria in humans, the most common being *Plasmodium falciparum*; others are *P. ovale, P. vivax, P. malariae*, and *P. knowlesi* (Lalloo et al., 2016). Mixed infections frequently go undetected unless molecular methods are used (Cnops et al., 2011). The reported proportion of mixed infections varies by geographic region from 1.2%–20% (Kotepui et al., 2020). In a recent study from Israel, among travelers returning from Sub-Saharan Africa, the incidence of mixed malaria infections was 3.7% (Manor et al., 2021).

Artemether-lumefantrine (AL) is a World Health Organization–recommended artemisinin-based combination therapy (ACT) for uncomplicated malaria due to any species (Anstey et al., 2015, Lalloo et al., 2016) with an efficacy of over 90% (Anstey et al., 2015). AL is assumed to cure mixed infections (Groger et al., 2018). Possible reasons for treatment failure include recrudescence related to poor compliance, insufficient drug concentrations, resistance to treatment, and in case of mixed infections, relapses of *P. vivax* or *P. ovale* hypnozoites (Rovira-Vallbona et al., 2019).

Case report

A Congolese man aged 24 years (BMI 20.7 kg/m^2) who lived in Belgium since childhood presented with fever at the University Hospital of Antwerp, Belgium on April 23, 2021, 7 days after returning from a three-week journey to Kinshasa, Democratic Republic of the Congo (DRC) to 'visit friends and relatives' (VFR) (Figure 1). He did not use mosquito bite prevention or malaria chemoprophylaxis. He had neither prior medical history nor previously documented malaria despite earlier visits to DRC. He complained of headache, anorexia, and abdominal pain for three days. His body temperature was 38.7°C, blood pressure was 104/55 mm Hg, and oxygen saturation was 99%.

Rapid diagnostic test targeting histidine-rich protein 2 and Plasmodium lactate dehydrogenase was positive. Blood smear confirmed ring-form *P. falciparum* trophozoites with 3646 asexual parasites/μL in a May-Grünwald-Giemsa–stained blood smear. Species-specific 4-primer real-time Polymerase chain reaction (PCR) detected *P. falciparum* deoxyribonucleic acid (Cnops et al., 2011). The patient had elevated C-reactive protein (CRP 106 mg/L) and thrombocytopenia (121 e^9/L), without liver or kidney dysfunction.

He was treated for uncomplicated *P. Falciparum* malaria in ambulatory care with 80/480 mg of oral AL at 0, 8, 24, 36, 48, and 60 hours. Although the treatment was not directly observed, the pa-

Abbreviations: ACT, Artemisinin combination therapy; AL, Artemether-lumefantrine; DRC, Democratic Republic of the Congo; P., Plasmodium; VFR, Visiting friends and family.

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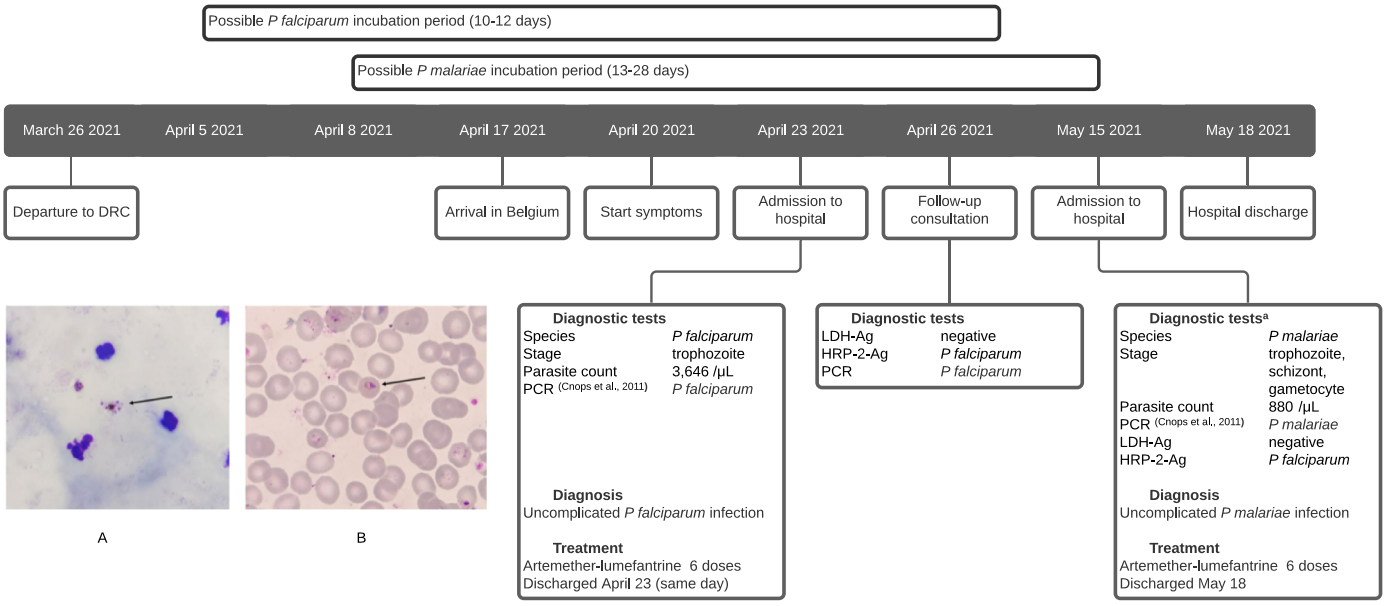

Figure 1. Graphical representation of the patient's course. P denotes plasmodium, DRC Democratic Republic of the Congo, PCR polymerase chain reaction, HRP-2 histidine-rich protein 2, LDH lactate dehydrogenase, Ag antigen.
Figure 1A – *P malariae* schizont (arrow) in a thick blood film, with 5 visible merozoites in an irregular cluster (May-Grünwald-Giemsa stain, x1000 magnification).
Figure 1B – Compact *P malariae* trophozoite with compact cytoplasm and a large chromatin dot (arrow) in a thin smear (May-Grünwald-Giemsa stain, x1000 magnification). Note the slightly reduced size of the infected red blood cell compared with noninfected cells.

tient reported full compliance. After three days, he reported clinical improvement. Control tests showed a negative thick blood film; repeat PCR detected only *P. falciparum* (Figure 1).

Three weeks after admission, the patient was hospitalized because of headache, abdominal pain, feverish feeling, and dehydration. There had been no new travel-related malaria exposure. Laboratory analyses showed elevated CRP (69 mg/L) and thrombocytopenia (146 e^9/L). A thin blood film and smear revealed trophozoites, gametocytes, and schizonts of *P. malariae* with 880 asexual parasites/μL but no *P. falciparum* parasites (see Figure 1A–1B). PCR confirmed the species identification. Because of this unexpected finding, a *P. malariae*-specific PCR was retrospectively performed on the samples obtained during the first malaria episode but remained negative. The patient was treated again with six doses of AL 80/480 mg over three days with rapid clinical and parasitological recovery. He was discharged after three days and remained free of symptoms during the 10-month follow-up.

Discussion

We report a case of two consecutive malaria episodes with different *Plasmodium* species in a patient after VFR-stay in DRC. The first episode with *P. falciparum* was successfully treated with AL. It is peculiar that three weeks later, *P. malariae* malaria became manifest, whereas it was not detected with initial tests. To our knowledge, this is the first report describing *P. malariae* malaria emerging this soon after a successfully treated *P. falciparum* in the absence of re-exposure.

First-line therapy of uncomplicated nonfalciparum malaria consists of chloroquine (when local susceptibility patterns of *P. vivax* permit) or any World Health Organization–recommended ACT including AL (Lalloo et al., 2016). AL is effective in clearing *P. malariae* parasites (Groger et al., 2018, Visser et al., 2014) and mixed infections (Dinko et al., 2013, Mombo-Ngoma et al., 2012, Visser et al., 2014). In the absence of malaria re-exposure, several explanations could account for the occurrence of *P. malariae* malaria shortly after the effective treatment of *P. falciparum*.

First, poor compliance to treatment or inadequate drug concentrations were considered unlikely because negative microscopy and clinical evolution showed that the *P. falciparum* infection was effectively cleared.

Second, *P. malariae* trophozoites could have been undetected during the first *P. falciparum* malaria due to low parasitemia, whereas a synchronous or coincident infection actually occurred (Collins and Jeffery, 2007). However, multiplex and post hoc singleplex PCR on samples of the first episode failed to detect *P. malariae* (Figure 1) despite the limit of detection being only 1 parasite/μL. AL should have effectively cleared all parasites as it did three weeks later. Although there is no robust data on AL efficacy against *P. malariae*, there have been few reports of failure (Grande et al., 2019, Rutledge et al., 2017). One hypothesis is that three-day AL might be suboptimal for the long erythrocytic life cycle (72 hours [Collins and Jeffery, 2007]) of this species (Rutledge et al., 2017). Furthermore, because the resistance of *P. falciparum* to AL is growing, a five-day regimen has been examined because longer treatment is considered to enhance efficacy (Tun et al., 2018). Whether a longer AL regimen could have prevented *P. malariae* three weeks later is uncertain.

Alternatively, in negative laboratory test results due to low parasitemia, *P. malariae* parasites could have been present in their prepatent phase at the time of the *P. falciparum* malaria, making them undetectable. This phase (the time until the first day of possible parasite detection) varies from 16–59 days (Collins and Jeffery, 2007). It consists of a pre-erythrocytic stage lasting between 13 and 28 days and erythrocytic multiplication in blood until patent parasitemia is reached (Collins and Jeffery, 2007). Thus, synchronous coinfection, with parasites at different stages of the life cycle, remains possible for the current presentation. The prepatent period and prolonged pre-erythrocytic stages of *P. malariae* are plausible explanations for the clinical course because AL does not affect the pre-erythrocytic stages (Calleri et al., 2013).

Finally, by analogy of *P. vivax* and *P. ovale*, an AL-unresponsive extraerythrocytic hypnozoite stage could explain the initial absence and later occurrence of *P. malariae* malaria. Although there is little

evidence to deny the existence of a quiescent liver stage, there is no solid demonstration of its existence (Collins and Jeffery, 2007, Grande et al., 2019 Rutledge et al., 2017).

The most likely scenario in our opinion is that *P. falciparum* and *P. malariae* infections occurred consecutively. The patient was probably infected with *P. falciparum* first and *P. malariae* later. Thus, *P. malariae* must have been present in the pre-erythrocytic stage during initial AL treatment. It is conceivable that *P. malariae* parasites could not be cleared, explaining failure of initial AL to clear all parasites (see Figure 1). The successful elimination of *P. malariae* with identical treatment later fits this theory.

Conclusion

We present a case of *P. malariae* malaria occurring three weeks after successful treatment of a *P. falciparum* infection with AL. Different possible explanations were reviewed. The longer prepatent period and prolonged pre-erythrocytic phase of *P. malariae* could explain why the first AL course did not prevent the second episode. Thus, caution is needed for a possible recurrence of (a different species of) malaria even after successful treatment.

Declaration of Competing Interest

All authors deny any financial or personal relationships with other people or organizations that could inappropriately have influenced (biased) the work.

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Ethical approval statement

The patient gave written informed consent for the publication of this case. All authors have read and complied with the policy of the journal on ethical consent as stated in the Guide to Authors. Approval was not required for this work.

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