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Multiple episodes of *Plasmodium malariae* despite antimalarial treatment: "Quartana te teneat"?

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Dear Editor

Plasmodium malariae, the quartan malaria parasite, is a neglected malaria parasite of humans. Both recrudescent-type and relapse-type long-latency recurrences have been previously reported but the biological mechanism underlying its persistence is still unknown [1]. Here, we report the case of woman from Montenegro in her 40's (BMI 23 kg/m^2), resident in Italy who acquired *P. malariae* infection while visiting the South Sudan and experienced two bouts of malaria attack after apparently initial successful treatment with chloroquine.

She sought care at Luigi Sacco University Hospital on November 5, 2022, with a 7-day history of febrile episodes (up to 39.5 °C) without other symptoms (Fig. 1). She had returned to Italy on October 8, after a 1-week trip to South Sudan from October 2 to October 8). She reported to have not taken anti-malarial chemoprophylaxis during the trip. Laboratory investigations showed absence of anemia (Hemoglobin, Hb, 13 g/dL), moderate thrombocytopenia (platelets 105,000/μL) and leukopenia (White Blood Cells, WBC, 3590/μL), increased levels of C-Reactive Protein (CRP, 147 mg/L) and lactate dehydrogenase (LDH, 257 U/L).

Given the travel history, the patient was tested for malaria: thick and thin blood smears stained with Giemsa showed the presence of *P. malariae* trophozoites; parasitemia was equal to 0.4 %. A Loop-Mediated Isothermal Amplification (LAMP) assay (Illumigene Malaria, Meridian Bioscience, Cincinnati, OH) resulted positive for *Plasmodium* spp. DNA, RDT (Paramax-3, Zephyr Biomedical Systems) detected *Plasmodium*-Pan antigen.

P. malariae species confirmation was performed by Real Time PCR (RT-PCR) through the RealStar® Malaria Screen & Type PCR Kit 1.0 RUO (altona Diagnostics GmbH, Hamburg, Germany). The RT-PCR Cycle threshold (Ct) value was equal to 25. The patient was hospitalized at the Infectious Diseases (ID) Department and treated with a standard regime of oral chloroquine (CQ) phosphate, at first 1000 mg (600 mg base), then 500 mg (300 mg base) 6 hours after the first dose and 500 mg (300 mg base) on the second and third days of treatment. The therapy was well tolerated and her clinical conditions rapidly improved. On November 9, 2022, peripheral blood smear was negative and the patient was discharged.

However, on February 6, 2023, she was readmitted at the Emergency Department (ED) of our hospital, presenting high fever along with persistent chills that started several days before. Blood examinations showed moderate anemia (Hb 11 g/dL), leukopenia (WBC 2360/μL), thrombocytopenia (platelets 59,000/μL), increased CRP (136 mg/L) and LDH (333 U/L). Peripheral blood smears and RT-PCR (Ct = 23) resulted again positive for *P. malariae*. Thin blood smear showed the presence of trophozoites, gametocytes and schizonts with a 0.2 % parasitaemia.

The patient conditions improved after treatment with chloroquine phosphate with the recommended scheme as previously indicated and she was discharged on February 9, 2023. On 13 February, during a follow-up visit, a peripheral blood smear tested negative for blood parasites.

The patient presented again at our ED on May 8, 2023 complaining of fever, arthralgia and myalgia. Laboratory examinations showed mild anemia (Hb 12 g/dL, HT 36 %), thrombocytopenia (platelets 130,000/μL) and leukopenia (WBC 3630/μL), a moderate increase in CRP (28.8 mg/L) and LDH (235 U/L). Rare trophozoites and gametocytes were observed on both thick and thin blood smears (parasitaemia <0.1 %). RDT for Malaria Pan antigen resulted negative whereas LAMP turned positive for *Plasmodium* spp. RT-PCR confirmed the presence of *P. malariae* DNA (Ct = 25). The patient was hospitalized once again at our ID department and at this time we decided to treat with dihydroartemisinin-piperaquine (DHA-PPQ, 320/40 mg) at the recommended regimen, 3 tablets once daily for 3 days. Clinical conditions improved and the patient was discharged on May 11, 2023. During the follow-up on May 26, 2023, blood examinations including Hb, WBC and platelets counts were unremarkable. Peripheral blood smear and LAMP resulted negative for *Plasmodium* spp. Up to October 10, 2023 (last follow-up) no further malaria attacks were recorded.

This report of a case characterised by two recurrent *P. malariae* episodes with apparent chloroquine treatment failure after directly observed and properly administered therapy in a non-endemic setting, where reinfection is not possible, highlights the poor knowledge of the biology of this parasite, almost one hundred and thirty-four years after its discovery (*Plasmodium malariae*, Feletti & Grassi 1889). Asymptomatic infections by *P. malariae* lasting for several decades have been described in people who had migrated to a malaria-free country or living in countries that became malaria-free [2,3]. Moreover, development of

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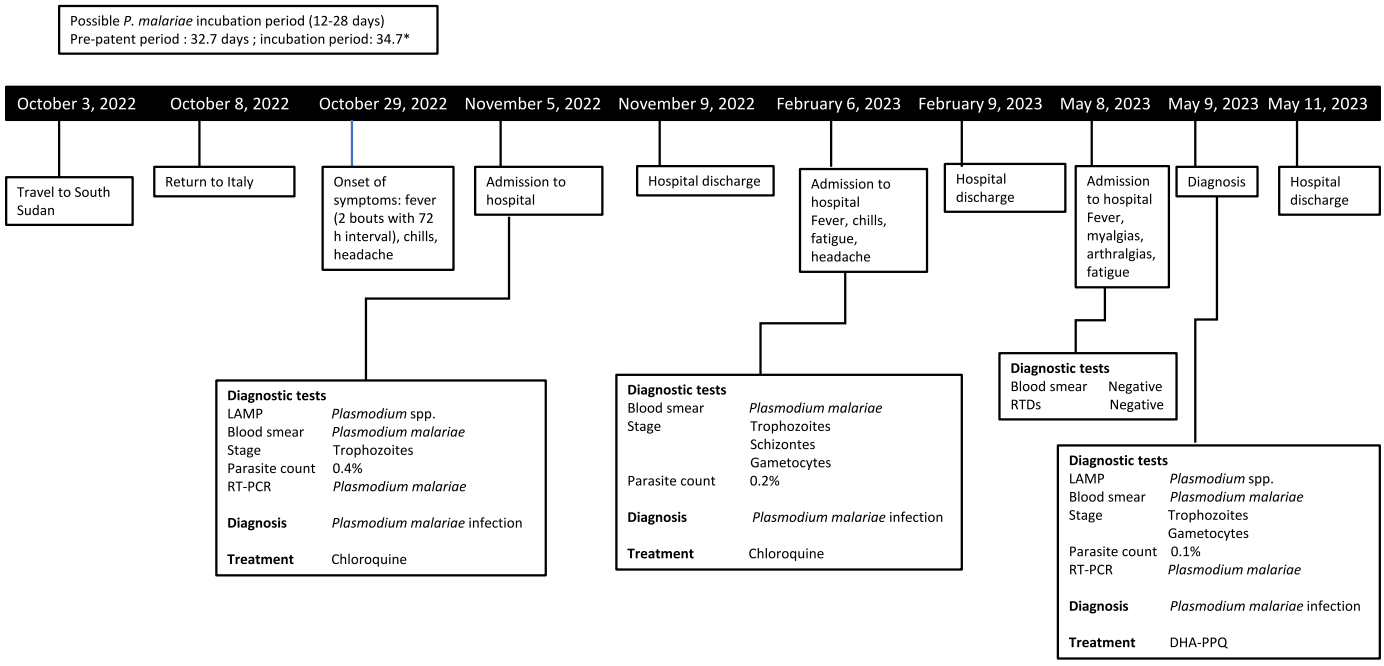

Fig. 1. Graphical representation of the patient's course.
RTDs, rapid diagnostic tests; LAMP, Loop Mediated Isothermal Amplification; RT-PCR, real-time polymerase chain reaction; DHA-PPQ, dihydroartemisinin-piperaquine- * These data are taken from artificially induced malaria data reported by Boyd (1948).

P. malariae infection following successful treatment of *P. falciparum* malaria has been also previously reported [4,5]. Furthermore, we have previously reported a case of *P. malariae* recurrence in which genotyping showed a substantial genetic diversity [6]. Unfortunately, in the present case we didn't have the chance of genotyping *P. malariae* strains responsible of the three documented attacks.

However, the exact mechanisms of persistence of *P. malariae* are still not elucidated because persisting dormant liver stages (i.e., hypnozoites) has never been demonstrated [1,2,6,7] although it is also true for the absence of proofs of excluding this possibility [8]. At variance with previous reports in the case we have described here an overlooked mixed *Plasmodium* infection was excluded by the use of molecular biology [4,5]. We cannot exclude the possibility that the first recrudescence in our patient can be ascribed to chloroquine resistance although for *P. malariae* this phenomenon have been reported only from Indonesia [9]. Inadequate drug absorption with suboptimal blood concentrations of chloroquine might be another explanation for this patient's recrudescent infection that cannot be excluded with certainty since no pharmacokinetic study was performed in our patient.

This case highlights the importance of testing, also in non-endemic regions, symptomatic patients with past history of *P. malariae* infection, in order to exclude long-latency recrudescence and drug-resistance. In conclusion, our case confirms the old Latin adage "quartana te teneat" (quartana fever is tenacious) for a neglected *Plasmodium* which deserves insightful studies to solve the mysteries about it [1].

Ethical approval

All data were anonymized. Patient consents have been obtained to include case details.

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CRediT authorship contribution statement

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Ivano Faggion: Investigation, Resources, Writing - Review & Editing.
Anna Gigantiello: Investigation, Resources.
Federica Salari: Investigation, Resources, Writing - Original Draft.
Fosca Niero: Investigation, Resources.
Simone Passerini: Investigation, Resources.
Chiara Mariani: Investigation, Resources.
Spinello Antinori: Investigation, Resources, Writing - Review & Editing, Supervision.
Maria Rita Gismondo: Writing - Review & Editing, Supervision.

Data availability statement

Data will provide by the corresponding author upon reasonable request.

Declaration of competing interest

All the Authors none to declare.

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