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*Case Report*

# Prozone-like phenomenon in travellers with fatal malaria: report of two cases

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## Abstract

Malaria diagnosis remains a concern in non-endemic countries, with rapid diagnosis being crucial to improve patients' outcome. Rapid diagnostic tests have high sensitivity but they also have flaws and false-negative results that might jeopardize malaria diagnosis. Some false-negative results might relate to a prozone-like effect. The authors describe two patients with false-negative rapid diagnostic tests in which a prozone-like effect might have been involved. The authors highlight that these tests should not be used without accompanying light microscopy observation of blood films and discuss potential benefits of using rapid diagnostic tests with more than one specific antigen for *Plasmodium falciparum*.

**Key words:** Malaria; rapid diagnostic tests; prozone effect; *Plasmodium.*

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## Introduction

Malaria diagnosis in non-endemic countries remains a concern with approximately 10,000 cases of imported malaria occurring each year in European countries [1]. Timely diagnosis and treatment are crucial to tackle the potentially devastating effects of the disease. Diagnosis of malaria by light microscopy examination of thin and thick blood films is still the gold standard [1], but rapid diagnostic tests (RDTs) are increasingly used both in non-endemic and endemic regions. These tests detect *Plasmodium* antigens in blood by antigen-antibody interaction on a nitrocellulose test strip and can target antigens specific of *P. falciparum* like the histidine-rich protein 2 (HRP-2) and lactate dehydrogenase (Pf-pLDH) or parasite antigens considered pan-specific (pLDH and aldolase). More than 200 tests are on the market, but only BinaxNow Malaria (Binax, Scarborough, USA) a combination assay with antibodies for detection of HRP2 and aldolase, has been approved by the US Food and Drug Administration [2]. Their global sensitivities are around 95% for detection of *Plasmodium falciparum*, however, false negative results may occur, with the majority of cases being related to low parasite densities (below 100 asexual parasites/μl or <0.002% of parasitized red blood cells (RBC) or non-falciparum-infections, for which the RDTs are less sensitive [3]. False-negative results have also been reported at high parasite densities, which may be explained by gene deletions, variation in antigenic structure or a prozone-like effect [4]. Prozone effect is defined as false-negative or false-low results in antigen-antibody immunological reactions, due to an excess of either antigens or antibodies [3].

We report two distinct cases of severe malaria in Portuguese travelers in which initial RDTs were negative for *P. falciparum* infection.

## Case Presentation

*Case Report 1*

A 53-year-old man whose medical history was remarkable for diabetes and a past myocardial infarction, presented with 5 days of fever, chills, headache and myalgia.

The patient returned from Angola five days previous, where he had lived for the past 3 years. He did not take malaria chemoprophylaxis, and previous malaria episodes were absent. At admission the vital

signs and physical exam were unremarkable and analytical workup revealed pancytopenia (Table 1); thin blood film revealed *P. falciparum* (2% parasitaemia) and RDT (BinaxNOW) was negative. He started intravenous quinine dihydrochloride and doxycycline. The next day, thin blood film was negative for *Plasmodium* and RDT turned positive (*P. falciparum* or mixed infection). He developed progressive hypoxemic respiratory failure and on hospital day 3 he required admission to an intensive care unit and invasive mechanical ventilation. Thoracic CT scan suggested malaria-related acute respiratory distress syndrome (ARDS). Blood and respiratory cultures were negative. Microscopy for malaria remained negative. Progressive deterioration was observed with a refractory hypoxemia and renal, hepatic and cardiovascular dysfunctions and despite appropriate medical therapy, including extracorporeal membrane oxygenation (ECMO) support, the patient died in intensive care unit (ICU) due to nosocomial sepsis with multi-organ failure on day 49.

*Case Report 2*

A 40-year-old man with controlled juvenile rheumatoid arthritis without medication, presented to the emergency room with 4 days of fever, myalgia and asthenia.

The patient returned from an 8-day working trip to Angola and reported compliance to malaria chemoprophylaxis with mefloquine. Fourteen days after arrival, high-grade fever (40º C), myalgia and asthenia were elicited. On the second day of symptoms, the patient went to his rheumatologist concerned with a possible rheumatoid arthritis flare. The next day, diarrhea began followed by jaundice and coluria. On emergency room arrival he was febrile and conscious. Vital signs were normal and physical examination was unremarkable except for hepatomegaly. He had thrombocytopenia and acute renal failure (see table 1). RDT (BinaxNOW) was negative for *P.falciparum* but a thin blood film revealed the presence of *P. falciparum* trophozoites with 30% parasitaemia.

In a few hours, rapid deterioration of neurological status was observed and invasive mechanical ventilation was required. Brain rain CT showed diffuse cerebral edema and cerebellar tonsils herniation. The patient was admitted to the intensive care unit for malaria treatment (intravenous quinine dihydrochloride plus doxycycline) and multiple organ support.

Despite adequate treatment and decreasing parasitaemia, bilateral fixed mydriasis was observed and brain CT revealed worsened cerebral edema. The patient died 48 hours after admission.

## Discussion

The two methods routinely used in our laboratory for malaria parasitological diagnosis are light microscopy and RDTs. The latter detects parasite-specific antigens or enzymes and some have a certain ability to differentiate species, being powerful tools to achieve a quick diagnosis both in endemic and non-endemic regions. However, RDTs have limitations: quantification of parasitaemia is impossible, there is a possibility of technical and procedural errors, HRP2-based tests may remain positive for several weeks after malaria treatment alongside with false negative results occurrence [1]. These false negative results can occur both for very low and high parasite densities. The latter can be related to the prozone effect. Indeed, there are some reports of this kind of phenomenon in malaria both *in vitro* and *in vivo*, in RDTs targeting HRP2 antigen [3-5] but not in other antigens like Pf LDH.

Light microscopy is still the gold standard for malaria diagnosis, so both negative and positive results should be confirmed with microscopy. There are several techniques to estimate parasitaemia and different stains can be used in light microscopy

**Table 1**. Laboratory data

| | **Case report 1** | | **Case report 2** | |
|---|---|---|---|---|
| | **Day 1** | **Day 2** | **Day 1** | **Day 2** |
| Thin blood film | *P. falciparum* 2% parasitemia | Negative | *P. falciparum* 30% parasitemia | *P. falciparum* < 1% parasitemia |
| RDT (Binax Now Malaria) | Negative | *P. falciparum* or mixed infection | Negative | Non-falciparum infection |
| Hgb g/dL | 10.5 | 10 | 12.2 | 10 |
| Platelet count (/μL) | 17 000 | 29 000 | 34 000 | 30 000 |
| Leukocytes (/μL) | 2270 | 3440 | 4 910 | 4070 |
| Creatinine mg/dL | 0.7 | 0.9 | 1.4 | 0.8 |

malaria diagnosis. Giemsa stain and determination of parasitaemia is performed by our lab in thin blood film by counting several hundred to 1000 RBCs, with the result being reported as the percentage of infected RBCs per 100 RBCs counted. Parasitaemia results are confirmed by at least two well-trained microbiologists of our hospital and the quality of light microscopy is assessed monthly, both by an internal procedure, and by the United Kingdom National External Quality Assessment Service for Microbiology.

In the two cases reported, there were initial negative RDTs that may be explained by a prozone-like effect. In case report 1, the initial parasitaemia was 2%, but considering the severity of the case, this parasitaemia could be an underestimation related to *P. falciparum*-infected erythrocytes adherence to microvascular endothelium and sequester. This might also raise the concern that prozone effect can occur for parasitaemias lower than those previously described [5].

In case report 2, the RDT had not detected *P.falciparum* antigens initially, despite the presence of high parasitaemia, in a patient with an autoimmune disease and under prophylactic mefloquine treatment. It seems that in this case the HRP-2 was not detected by the test. However, the presence of a prozone-like effect is likely to have occurred in both cases regarding the relatively high parasite densities, this effect could not be properly confirmed because a repeated test with diluted samples was not performed. Although a device operator error was unlikely, we cannot exclude that the two negative tests resulted from improper conduct of RDTs. Another possible explanation in case report 2 is the HRP-2 gene absence or genetic polymorphism. Genetic variation of the HRP-2 gene is common but the diversity of proteins is not a major cause of variation in sensitivities of RDTs [6]. HRP-2 gene deletion is less common but has already been described in isolates from Peru, Brazil, India and Mali [6]. HRP-2 genotyping is not performed by our laboratory, so we cannot completely exclude HRP-2 genetic variation as a cause of false-negative results.

Whatever the explanation for the false-negative results may be, if the RDTs were used as the only diagnostic tool the malaria diagnosis could have been missed, jeopardizing the prompt initiation of an adequate therapy. Other available RDTs target different *P. falciparum* antigen, namely Pf LDH and for those tests the prozone effect was not described. However, RDTs targeting HRP-2 have some advantages over those targeting Pf LDH, like higher sensitivity and higher heat resistance [1]. Probably RDTs combining both antigens can eliminate the shortcomings presented by each other, when used alone.

These two cases highlight that, although false negative RDTs are rare, they can impair a timely malaria diagnosis if RDTs are used alone. Although RDTs are powerful, highly efficient and continuously evolving tools, at present, they cannot be used as an alternative to microscopy and they constitute an adjunct that can help orienting the diagnosis, particularly when a skilled microscopist is not available to identify *Plasmodium* species.[1]

In addition to adequate diagnosis and timely treatment, preventive measures are essential in order to avoid severe imported malaria. Together with bite avoidance measures, pre-travel consultation and adherence to an effective regimen of chemoprophylaxis are essential for protection against malaria. Currently recommended chemoprophylaxis includes mefloquine, atovaquone plus proguanil and doxycycline, with all regimens showing efficacy rates higher than 90% in clinical trials [7]. However, compliance with such regimens is still sub-optimal [8]. The patient of case report 2 developed severe malaria despite chemoprophylaxis with mefloquine raising the question about chemoprophylaxis efficacy outside clinical trials, although adequate compliance to the prophylaxis regimen could not be assessed. In fact, a retrospective study by Zuckerman *et al.* [7] in United Kingdom travellers showed that efficacy of mefloquine as chemoprophylaxis was lower than the regimen containing atovaquone plus proguanil with the differences being attributed to compliance issues related to mefloquine side effects. Additional data is needed to clarify possible differences in efficacy of chemoprophylaxis regimens and to help clinicians to choose the best option for each patient.

Treatment of severe malaria involves the use of intravenous antimalarial drugs and providing adequate support to deal with possible organ dysfunctions. The two patients reported here were treated in an intensive care unit dedicated to infectious diseases that has a large experience in severe malaria management [9]. The two patients were treated with intravenous quinine dihydrochloride plus doxycycline because in Portugal intravenous artesunate, the first choice for severe malaria treatment according to the World Health Organization guidelines [10], is unavailable. Higher efficacy of intravenous artesunate when compared to quinine containing regimens in the treatment of severe

malaria is well documented [11] and can lead to significant reductions in mortality.

Severe imported malaria continues to be an important problem in western countries and better preventive strategies, accurate and timely diagnosis and worldwide availability of the best treatment options are needed in order to tackle this potential fatal disease. This report also highlight the importance of clinical suspicion and the central role of light microscopy in the accurate diagnosis of malaria.

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