False-positive rapid diagnostic test for malaria in new world cutaneous leishmaniasis: a tale of two travelers

Rebecca Unterborn, Jose Henao-Cordero, Arianna Kousari, Poornima Ramanan, Carlos Franco-Paredes and Nancy Madinger

Abstract: We report two immigrants from Cuba seen in a US travel clinic with a confirmed diagnosis of cutaneous leishmaniasis in whom we also suspected malaria co-infection. Both individuals likely acquired leishmaniasis in the Darien Gap region of Panama during their migratory path to the United States. As part of their clinical workup to rule out malaria, a rapid malaria antigen testing for *P. falciparum* was obtained and reported positive in both patients, However, both a qualitative reverse transcription-polymerase chain reaction (RT-PCR) for *Plasmodium falciparum* in blood and repeated thick-and-thin smear direct microscopy were negative in both, deeming the rapid malaria test as a false-positive. Thus, confirmation of malaria in travelers requires thick-and-thin film microscopy. Clinicians should be aware of the growing recognition of the possibility of false-positive malaria rapid diagnostic tests in those with some forms of leishmaniasis

Keywords: cross-reactivity, cutaneous leishmaniasis, leishmaniasis, malaria, *Plasmodium falciparum*

Introduction

Cutaneous leishmaniasis is a major neglected tropical disease (NTDs), endemic in over 98 countries and an estimated 350 million people are at risk. Different subspecies can cause diverse clinical manifestations. In the Americas (New World), most cases are caused by infection with *Leishmania mexicana* species complex, *Leishmania amazonensis*, and the *Leishmania Viannia* subgenus complex. Infection with *Leishmania panamensis* manifests as either cutaneous leishmaniasis, diffuse cutaneous leishmaniasis, or cutaneous leishmaniasis with early mucosal involvement. Neglected tropical diseases do not occur in isolation: at-risk individuals may have concomitant NTDs co-infections and may be poly parasitized. For example, a recent study from Southern Ghana demonstrated a high prevalence of *Plasmodium falciparum* and *Schistosoma mansoni* infection.

Case descriptions

**Case 1**

A 24-year-old Cuban immigrant came in with worsening nonhealing lower extremity ulcers despite oral antibiotic therapy and wound care. The patient reported these lesions gradually grew with subsequent ulceration over the course of 1 to 2 months. He recently arrived at the U.S. seeking asylum. He first noticed them while traveling in the jungle between Panama and Colombia (Darien Gap region). On exam, the ulcerative lesion measured 4 cm on the dorsum of the right foot. We identified a second 3.5 cm ulcer on the lower anterior shin (Figure 1). There was no evidence of mucosal or visceral involvement on initial physical exam.

**Case 2**

A 47-year-old man who was a traveling companion of the patient described above presented with
a total of 10 lesions on bilateral upper (Figure 2) and lower extremities and flank, all >1 cm with the largest measuring 5 cm. Tissue samples were sent to the Centers for Disease Control and Prevention, Atlanta, GA, USA, for confirmation of diagnosis. Samples from our two patients were positive for *Leishmaniasis* by immunohistochemistry and histopathologic evidence of amastigotes in both cases. Both individuals likely acquired leishmaniasis in the Darien Gap region of Panama during their migration to the United States. As part of their workup to detect other potential tropical infections, we obtained rapid malaria diagnostic tests (RDTs) in both patients. Both patients had positive RDTs for *P falciparum* but no evidence of parasites.

**Discussion**

We report two Cuban travelers seen in a US travel clinic with a confirmed diagnosis of cutaneous leishmaniasis in whom we also suspected malaria co-infection. The diagnosis of leishmaniasis in both of our patients was made in both patients by immunohistochemical confirmation and by visualization of amastigotes in biopsies of cutaneous lesions. Both individuals likely acquired leishmaniasis in the Darien Gap region of Panama during their migration to the United States. As part of their workup to detect other potential tropical infections, we obtained rapid malaria diagnostic tests (RDTs) in both patients. Both patients had positive RDTs for *P falciparum* but no evidence of parasites.

Malaria rapid diagnostic tests to detect *P falciparum* utilize histidine-rich protein 2 (PfHRP2) and pan, *P falciparum* or *P vivax*-specific *Plasmodium* lactate dehydrogenase to differentiate *P falciparum* from non-*P falciparum* infections. Obtaining RDTs provide initial information that assist clinicians in starting empiric malaria therapy. However, it is important to consider that the diagnostic value of these tests is compromised by the occurrence of false-positive results that require
confirmation with direct microscopy of peripheral blood smears. There are a few conditions to consider that may produce false-positive RDTs. For example, patients with detectable rheumatoid factor may have false-positive malaria RDT. In addition, false-positive malaria RDT results have also been reported in persons with Human African Trypanosomiasis (HAT), acute schistosomiasis, and more recently in a returning traveler with enteric fever due to Salmonella typhi. False-positive reports have been documented, specifically for the P. falciparum band on the rapid malarial antigen test in patients with positive Leishmania seropositivity. Conversely, cross-reactivity between Plasmodium and leishmaniasis has been observed with false-positive leishmaniasis antigens by immunofluorescent antibody (IFA) testing, in patients with malaria. Interestingly, there are no documented antigens shared between Plasmodium and Leishmania spp., so this relationship is poorly understood.

Conclusions
Clinicians should be aware of the growing recognition of the possibility of false-positive malaria rapid diagnostic tests in those with some forms of leishmaniasis, as well as other NTDs such as HAT and schistosomiasis. This consideration is particularly important for tropical regions of the world where there is no quality-assured microscopy available to confirm malaria diagnosis. In other settings, the use of rapid diagnostic tests may assist clinicians in initiation early therapy. However, quality-assured examination by direct microscopy of blood smears to detect Plasmodium parasites need to be performed as it is the gold standard for confirming a diagnosis of malaria.

Consent for publication
Verbal informed consent for publication was obtained from both patients.

Author contribution(s)
Rebecca Unterborn: Investigation; Writing – original draft.
Jose Henao-Cordero: Conceptualization; Investigation; Project administration; Supervision; Visualization; Writing – original draft; Writing – review & editing.
Arianna Kousari: Writing – original draft; Writing – review & editing.

Poornima Ramanan: Supervision; Validation; Writing – review & editing.
Carlos Franco-Paredes: Supervision; Writing – original draft; Writing – review & editing.
Nancy Madinger: Supervision.

ORCID iDs
Jose Henao-Cordero https://orcid.org/0000-0002-1947-8675
Carlos Franco-Paredes https://orcid.org/0000-0001-8757-643X

Funding
The authors received no financial support for the research, authorship, and/or publication of this article.

Conflict of interest statement
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References
1. Mann S, Phupitakphol T, Davis B, et al. Case report: cutaneous leishmaniasis due to Leishmania (Viannia) panamensis in two travelers successfully treated with miltefosine. Am J Trop Med Hyg 2020; 103: 1081–1084.
2. Mann S, Frasca K, Scherrer S, et al. A review of leishmaniasis: current knowledge and future directions. Curr Trop Med Rep 2021; 8: 121–132.
3. Olivo Freites C, Gundacker ND, Pascale JM, et al. First case of diffuse leishmaniasis associated with Leishmania panamensis. Open Forum Infect Dis 2018; 5: ofy281.
4. Hotez PJ, Molyneux DH, Fenwick A, et al. Incorporating a rapid-impact package for neglected tropical diseases with programs for HIV/AIDS, tuberculosis, and malaria. PLoS Med 2006; 3: e102.
5. Akosah-Brempong G, Attah SK, Hinne IA, et al. Infection of Plasmodium falciparum and helminths among school children in communities in Southern and Northern Ghana. BMC Infect Dis 2021; 21: 1259.
6. Gatton ML, Ciketic S, Barnwell JW, et al. An assessment of false positive rates for malaria rapid diagnostic tests caused by non-Plasmodium
infectious agents and immunological factors. *PLoS ONE* 2018; 13: e0197395.

7. Gillet P, Mumba Ngoyi D, Lukuka A, *et al.* False positivity of non-targeted infections in malaria rapid diagnostic tests: the case of human African trypanosomiasis. *Plos Negl Trop Dis* 2013; 7: e2180.

8. Leshem E, Keller N, Guthman D, *et al.* False-positive Plasmodium falciparum histidine-rich protein 2 immunocapture assay results for acute schistosomiasis caused by *Schistosoma mekongi*. *J Clin Microbiol* 2011; 49: 2331–2332.

9. Meatherall B, Preston K and Pillai DR. False positive malaria rapid diagnostic test in returning traveler with typhoid fever. *BMC Infect Dis* 2014; 14: 377.

10. Kohanteb J and Ardehali S. Cross-reaction of sera from patients with various infectious diseases with *Leishmania infantum*. *Med Princ Pract* 2004; 14: 79–82.