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The Brief Case: False-Positive Rapid Malaria Antigen Test Result in a Returned Traveler

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CASE

A previously healthy 43-year-old female presented to her primary care physician with a 13-day history of recurrent nightly fevers (100°F to 101°F), nonproductive cough, and respiratory congestion. She was prescribed azithromycin, albuterol, and benzonatate. She presented to the emergency center 2 days later without improvement and at that time reported night sweats. She had recently traveled to Thailand, Laos, Cambodia, and the United Arab Emirates. The nightly fevers began 11 days after returning home. She acknowledged being bitten by mosquitoes throughout her travels and did not take malaria prophylaxis.

Upon presentation, she was febrile (100.4°F) and tachypneic; right basilar rhonchi were noted. Chest X ray demonstrated multiple nodular lower right lobe airspace opacities. Laboratory evaluation showed mild leukocytosis with absolute neutrophilia, thrombocytosis, and mildly elevated alkaline phosphatase and alanine aminotransferase levels. Meropenem and doxycycline were started for community-acquired pneumonia and melioidosis, given her travel history to Southeast Asia. Additionally, atovaquone-proguanil was started, and a blood parasite examination was performed, including microscopy (thick and thin blood films) and a BinaxNOW malaria antigen test (BinaxNOW; Alere, Scarborough, ME). BinaxNOW was weakly positive for *Plasmodium falciparum* antigen and negative for pan-malaria antigen (Fig. 1). No parasites were identified by microscopy. Over the next 24 h, two additional blood parasite examinations were performed, both yielding results identical to the initial results. Her condition rapidly improved; all bacterial cultures were negative, and she completed a 3-day course of atovaquone-proguanil. Her antibacterial therapy was changed to oral levofloxacin to complete a 5-day course of therapy.

Discussion with infectious disease specialists highlighted that parasites may have been undetectable by microscopy, as the patients' previous course of azithromycin may have had limited activity against *P. falciparum*. Malaria real-time PCR was performed at an outside reference laboratory, and results were negative. These findings suggested a false-positive result for malaria antigen. She was further evaluated for autoantibodies known to cause interference with malaria antigen tests; tests for rheumatoid factor, cyclic citrullinated peptide, and anti-double-stranded DNA were all negative, and a test for anti-nuclear antibody was weakly positive (titer, 1:160; speckled pattern). These results essentially ruled out common autoantibodies as the source of the false positivity. By PCR, culture, and serologic techniques, the following other infectious etiologies were excluded: influenza virus, parainfluenza virus, respiratory syncytial virus, human metapneumovirus, rhinovirus, adenovirus, coronaviruses (including Middle East respiratory syndrome coronavirus), HIV, *Mycobacterium tuberculosis* (QuantiFERON Gold),

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For answers to the self-assessment questions and take-home points, see page 2560 in this issue (https://doi.org/10.1128/JCM.02349-16).

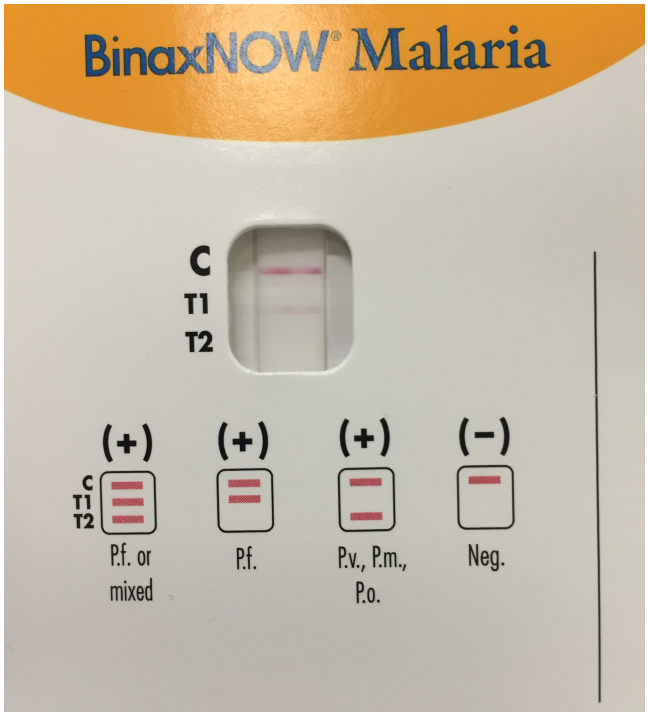

FIG 1 BinaxNOW malaria test results showing a weakly positive *Plasmodium falciparum*-specific antigen result (histidine-rich protein 2; T1 band), a negative pan-malaria antigen result (aldolase; T2 band), and a valid control line (C band). P.f., *Plasmodium falciparum*; P.v., *Plasmodium vivax*; P.m., *Plasmodium malariae*; P.o., *Plasmodium ovale*.

Histoplasma (serum/urine antigen), *Leptospira*, *Schistosoma*, *Strongyloides*, and *Legionella* and *Streptococcus pneumoniae* (urinary antigens). The patient did test positive for *Mycoplasma pneumoniae* by PCR.

It is known that infection by *Mycoplasma pneumoniae* can induce cold autoantibody formation. Multiple blood bank techniques were performed to determine whether a cold agglutinin was present. First, an initial gel antibody screening, performed at 37°C, was negative. Subsequently, an all-phase, tube antibody screen and autocontrol antibody screen were performed, which demonstrated room temperature, immediate spin pan-reactivity in the tube antibody screen and weak reactivity in the autocontrol antibody screen. The patient's specimen did not react at other phases of assessment (i.e., at physiologic temperature or with antiglobulin). Then, using all-phase tube testing, one adult I-antigen-negative panel cell was challenged and did not react at room temperature, with an immediate spin reading. Lastly, the patient's plasma was tested against three fetal cord red blood cell specimens (i.e., I-antigen negative) using the all-phase tube methodology. None of these fetal cells reacted with the patient's plasma. Overall, these testing pattern data support the presence of a cold autoantibody with probable I-antigen specificity, which might explain the patient's initial false-positive test.

DISCUSSION

Malaria continues to be a worldwide concern. In 2013, the Centers for Disease Control and Prevention received 1,727 reports of malaria, an increase of 2% over the prior year (1). Over the last 4 decades in the United States, there has been a continuous upward trend in reported cases of malaria due to more frequent travel to areas where malaria is endemic without proper preventative measures, most commonly lack of chemoprophylaxis (1).

The diagnosis of malaria should be considered in all febrile individuals who have traveled to areas of endemicity, as prompt identification of those patients is necessary to begin appropriate therapy and reduce complications (1). The gold standard for malaria

diagnosis remains light microscopy (thick and thin blood films) for identification and parasite quantification (1, 2). However, microscopy has limitations. It is labor-intensive and time-consuming, and it is difficult to maintain personnel proficiency (2, 3). However, other detection modalities are available, including rapid diagnostic tests and PCR.

Numerous malaria rapid diagnostic tests (MRDTs) are available worldwide, but only BinaxNOW is FDA approved for use in the United States (2). This is a qualitative test utilizing lateral-flow immunochromatography to detect malaria-specific antigens (1–3). In brief, the manufacturer provides a nitrocellulose membrane impregnated with monoclonal (capture) antibodies for *Plasmodium falciparum*-specific antigen (histidine-rich protein 2; HRP-2), pan-malaria antigen (aldolase), and an internal control (2–4). Aldolase antigen detects multiple *Plasmodium* species (*falciparum*, *vivax*, *ovale*, *malariae*) but cannot provide species-level identification (2, 3). A second set of mobile-phase (signal) antibodies conjugated with colloidal gold migrate with the sample via capillary action (2). During migration through the test strip, the sample binds to the internal control and HRP-2 and/or aldolase antigen, creating a visual result (2). The test is interpreted as positive or negative based on the presence or absence of a pinkish purple line within the respective areas of the test window (3). A negative result produces only a line within the control region (designated "C"); the absence of this line indicates an invalid test. A positive test result includes the presence of the control line in addition to HRP-2 (designated "T1") and/or aldolase (designated "T2") (2). For example, positivity for T1 alone indicates *P. falciparum* infection. Aldolase is not as sensitive as HRP-2 and may be negative for patients with low-level parasitemia. Positivity for T2 alone indicates infection with *P. vivax*, *P. ovale*, or *P. malariae*. Positivity for T1 and T2 indicates infection by *P. falciparum* alone or a mixed infection (*P. falciparum* along with another *Plasmodium* species). Other MRDTs are available outside the United States and utilize different combinations of detection antigens, including HRP-2, aldolase, *Plasmodium falciparum* lactate dehydrogenase (PfLDH), or *Plasmodium* lactate dehydrogenase (PLDH). Positivity for PLDH is synonymous with aldolase positivity, indicating infection with any type of *Plasmodium* species, whereas positivity for PfLDH is specific to *Plasmodium falciparum*.

In general, MRDTs are simple to perform and facilitate the prompt identification of infected patients, facilitating the appropriate use of antimalarial medications (2, 3). However, there are limitations of this technology, including the inability to quantify parasite load (initially or during therapy) or distinguish between mixed infections; therefore, microscopy and PCR methods are still needed (1, 3). In addition, false-negative results have been reported with low parasite levels (1–3). BinaxNOW sensitivity decreases as the level of parasitemia decreases; sensitivity for *Plasmodium falciparum* is >99% (>1,000 parasites/μl) and 54% (0 to 100 parasites/μl) and even lower for *P. vivax* infections at 94% (>5,000 parasites/μl) and 24% (100 to 500 parasites/μl) (3). This observation is also relevant to other MRDTs (2). Therefore, if there is a high clinical suspicion for *Plasmodium* infection and MRDTs are negative, reflex testing with microscopy and/or PCR is warranted.

In addition, false-positive MRDT results have been reported in cases of chronic hepatitis C, toxoplasmosis, human African trypanosomiasis, dengue, leishmaniasis, Chagas disease, and schistosomiasis (3, 4). The most common cause of false positives is attributed to rheumatoid factor (RF) (3, 4). Other heterophile antibodies should be considered potential causes of false-positive results in all immunoassays (5). A heterophile antibody is a naturally occurring antibody/autoantibody demonstrating reactivity to poorly defined antigens or an interfering endogenous antibody that reacts with immunoglobulins from two or more species (e.g., mouse) (5). Heterophile antibodies have IgG or IgM specificity and cause interference with conventional two-site immunoassays (like BinaxNOW) by binding to either the capture or the signal antibody (5). Lee and colleagues recently evaluated four different rapid malaria diagnostic tests, including BinaxNOW, for false-positive reactions in patients with RF (4). BinaxNOW demonstrated the highest false-positive percentage (13%), observed with both the HRP-2 and aldolase antigens (4). The BinaxNOW package insert states that testing was performed on 116 specimens to determine interference from other medical conditions,

including the presence of RF, antinuclear antibody, systemic lupus erythematosus, and human anti-mouse antibody (HAMA), and only 5 positive results (4 for RF and 1 for HAMA) were reported (3).

Our patient had a *Mycoplasma pneumoniae* infection, which led us to further investigate the cause of the false-positive result. *Mycoplasma pneumoniae* infections are known to be associated with the development of an auto-anti-I antigen (6). The I antigen is located on the surfaces of red blood cells as a polyvalent, branched glycan and is derived from the linear, nonbranched i antigen (6). Neonates are unable to convert i antigen to I antigen initially, but most children express a normal adult I^+ phenotype by 2 years of age (6). Auto-anti-I antigen is typically IgM and shows reactivity at 4°C; it is commonly found in normal adults and is usually of no clinical significance (6). However, patients infected with *Mycoplasma pneumoniae* may develop a potent auto-anti-I antigen, which activates complement and may be associated with transient hemolysis or cold agglutinin syndrome (6). Patients with high titers of auto-anti-I antigen are detected by strong agglutination reactions to adult (I^+) red blood cells at room temperature (i.e., antibody screening panels at immediate spin) and show no or weak reaction when tested against fetal (i^+) cord red blood cells (6). Given that the BinaxNOW test is performed at room temperature, we speculate that auto-anti-I antigen likely caused the false-positive BinaxNOW result.

To our knowledge, this is the first reported case of a false-positive BinaxNOW malaria test associated with an auto-anti-I antigen in a patient with concurrent *Mycoplasma pneumoniae* infection. Due to inherent test limitations and the consequences of missing or overdiagnosing malaria infection, it is necessary to microscopically confirm all rapid malaria antigen tests results.

SELF-ASSESSMENT QUESTIONS

1. In addition to performance of a rapid diagnostic test for malaria, which of the following is most appropriate to be performed?
    A. No further testing is required
    B. Real-time PCR
    C. Serology
    D. Blood film examination
2. Which of the following is a benefit of rapid diagnostic tests for malaria?
    A. The ability to detect low levels of parasitemia
    B. The ability to differentiate between non-*falciparum Plasmodium* species
    C. The ability to monitor therapy responses
    D. The ability to rapidly screen patients for malarial infection
3. Which of the following is correct regarding blood film microscopy?
    A. It has decreased sensitivity compared to those of rapid diagnostic tests for malaria
    B. Maintaining personnel proficiency is difficult
    C. Minimal technical labor is required
    D. Test results are rapid

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