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Case report

Cryptococcal pneumonia and meningitis in a renal transplant recipient with a false negative serum cryptococcal antigen due to postzone phenomenon

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ABSTRACT

Cryptococcal infection can cause significant morbidity and mortality in immunocompromised patients. We present a patient who was diagnosed with cryptococcal meningitis and pulmonary disease in the setting of a history of renal transplantation. The diagnosis was made based on growth of *Cryptococcus neoformans* in blood cultures and identification of cryptococcal antigen (CrAg) in cerebral spinal fluid (CSF) using a lateral flow assay (LFA). Our case is unique since the initial serum CrAg was falsely negative due to excess cryptococcal antigen preventing the formation of antigen-antibody complexes, referred to as the postzone phenomenon. This phenomenon has been reported on CSF samples but rarely reported on serum samples in patients without an HIV diagnosis.

Introduction

Cryptococcosis is an infection caused by *Cryptococcus neoformans* or *C. gattii*. *Cryptococcus* is a dimorphic fungus with a worldwide distribution, with majority of the global population having been exposed, but never developing clinically apparent illness. The majority of those who experience symptomatic illness are immunocompromised [1]. Typically, cryptococcal infections cause pulmonary or central nervous system disease. The gold standard for diagnosing cryptococcal infections is fungal culture from a clinical specimen. However, culture can take several days to demonstrate growth of *Cryptococcus*, thereby delaying the diagnosis and appropriate management [2]. A lateral flow assay to detect cryptococcal antigens (CrAg) is frequently used to screen for infection and can be performed using serum and cerebrospinal fluid. The LFA is a cost-effective, rapid test shown to be sensitive for *Cryptococcus* species detection [3]. We report a case of a renal transplant recipient with cryptococcemia, cavitary cryptococcal pneumonia and cryptococcal meningitis who had a negative serum LFA antigen test as a result of excessive CrAg.

The case

51-year-old man with history of end stage renal disease status-post living donor renal transplantation in January 2021 presented to our hospital in October 2021 with jaw pain and chills, associated with night sweats, nausea, vomiting, myalgias, and headaches. He had a long-standing history of odontalgia from a cracked wisdom tooth and recurrent urinary tract infections. His home medications included tacrolimus, mycophenolate, and prednisone. On initial evaluation, the patient was afebrile at 37 °C, tachycardic at 106 beats per minute, and hypertensive at 230/104 mmHg. Laboratory studies showed leukopenia (white blood cell (WBC) count of 4.0 × 10^3 cells/μL), creatinine of 1.2 mg/dL, C-reactive protein elevated at 222.5 mg/dL, and lactate dehydrogenase elevated at 649 units/L. Urinalysis was unremarkable.

Computed tomography (CT) scan of the chest revealed multiple cavitary pulmonary nodules (Image 1-3). Maxillofacial CT scan noted dental disease with no acute process. Blood cultures were obtained and he was started on vancomycin and meropenem. Bronchoscopy performed on admission day five was grossly normal and bronchioalveolar lavage (BAL) was performed. Serum CrAg testing on admission was negative, but blood culture reported growth of yeast on admission day

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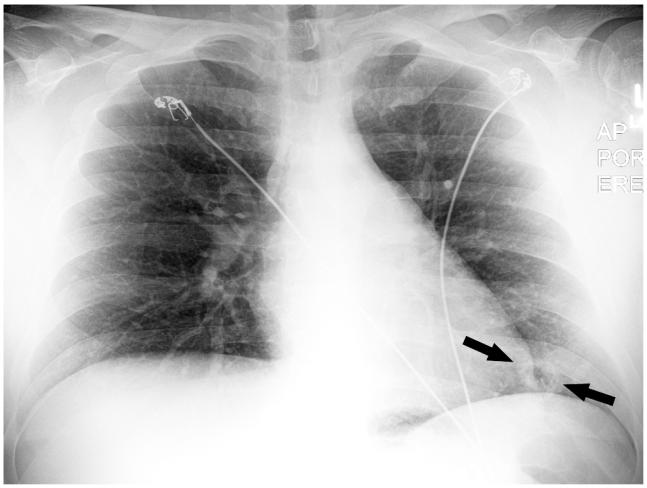

Image 1. Portable frontal chest radiograph shows a cavity in the left lower lung (black arrows).

six. The patient was started on liposomal amphotericin B 4 mg/kg IV daily and flucytosine 25 mg/kg orally four times daily (Images 2 and 3).

Lumbar puncture was performed on admission day nine with an opening pressure of 23 mmHg. CSF analysis showed WBC count of 1 cells/μL (2% neutrophils, 47% lymphocytes, 51% monocytes), elevated glucose of 110 mg/dL, and elevated protein of 50 mg/dL. A multiplex polymerase chain reaction (PCR) test performed on the CSF (Biofire® FilmArray® Meningitis/Encephalitis Panel, Biomerieux, Salt Lake City, Utah) was negative for all organisms, including *Cryptococcus* spp. However, the CSF CrAg titer was positive at a 1:1 dilution. The BAL culture grew *C. neoformans* after six days incubation. Given prior negative serum CrAg in the setting of cryptococcal meningitis, ten total dilutions were performed on serum, resulting in a positive serum CrAg with a titer of 1:2560.

Due to an increase in the patient's creatinine to 1.6 mg/dL, the flucytosine was discontinued after five days. The patient completed two weeks of amphotericin B before transitioning to oral fluconazole. Repeat chest CT scan on day 15 showed increased inflammation around the cavitary lung lesion, but likely represented an immune reconstitution inflammatory reaction and not treatment failure. The patient's renal function normalized. He was discharged on eight weeks of fluconazole 800 mg daily and remains on 400 mg daily maintenance therapy with plans to taper, based on repeat serum CrAg titers.

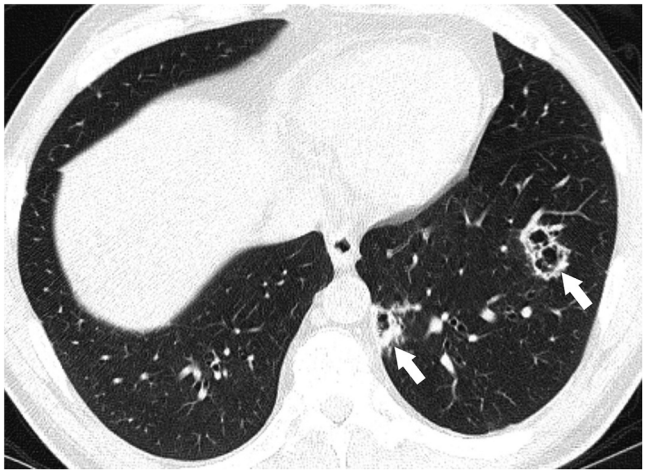

Image 2. Axial noncontrast CT shows multiple, clustered and irregular cavities in the left lower lobe (white arrows).

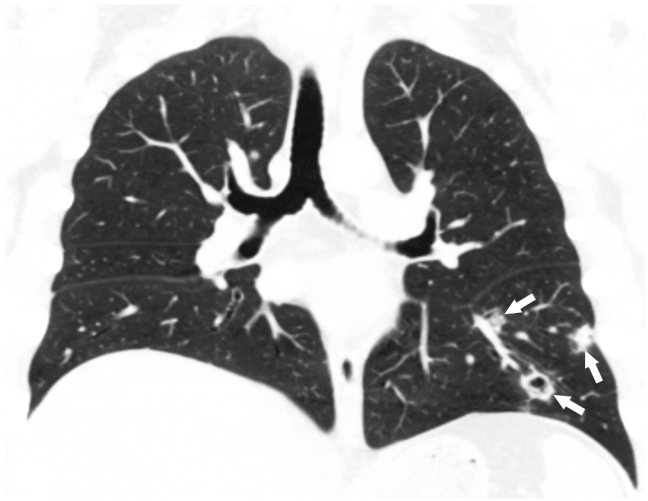

Image 3. Coronal reformatted noncontrast CT shows cavities and nodular opacities in the left lower lobe (white arrows).

Discussion

Diagnosis of cryptococcal meningitis can be undertaken using India Ink staining, culture, PCR, and CrAg detection. The CSF meningitis/encephalitis (M/E) PCR panel has a lower sensitivity than the cryptococcal LFA and can yield false negative results. In a retrospective analysis of 1384 CSF specimens tested, the M/E PCR panel detected *Cryptococcus* in 32 specimens while the CSF CrAg was positive in 37 [4]. The sensitivity of the M/E PCR was 83.8% compared with CSF CrAg testing, although all five CrAg positive, M/E PCR negative specimens were obtained from patients who were previously treated for cryptococcal meningitis.

In 2009, the LFA was introduced as a new option for the diagnosis of cryptococcal disease. The test offered multiple advantages: a 10-minute turnaround time, stability at room temperature and need for minimal laboratory equipment. These characteristics facilitate more rapid diagnosis of cryptococcal disease in resource limited countries. In a meta-analysis reviewing 12 studies evaluating the sensitivity and specificity of LFA on serum and CSF specimens for cryptococcal diagnosis, the pooled sensitivity and specificity was 97.6% and 98.1%, and 98.9% and 98.9% for serum and CSF specimens, respectively [5].

The cause of our patient's discrepant results is related to the postzone phenomenon, where the false negative result is paradoxically caused by an antigen excess in relation to antibodies. This antigen excess prevents antibody-antigen crosslinking, which is necessary for a positive result on the LFA. Although this phenomenon has been reported in CSF specimens, it has rarely been reported in serum samples [6].

As noted, the CrAg LFA is highly sensitive, and there have been multiple reports of false negative CrAg attributed to the post-zone effect. This should not be mistaken for a prozone effect, which is caused by excess antibody in relation to antigen. In one study evaluating diagnosis of cryptococcal meningitis using LFA on CSF, a 91% sensitivity in undiluted samples was found, while 100% sensitivity was noted after CSF sample dilution. In this protocol, the samples selected for dilution were those with negative LFA results and yeast present on Gram staining [7]. Similar results have been found in multiple case reports, highlighting the consideration of sample dilution in patients with findings concerning for cryptococcal infection.

Conclusion

We present an immunocompromised patient with disseminated cryptococcal infection who initially had a negative serum CrAg result, which subsequently became positive after ten dilutions confirming a

postzone phenomenon as the cause of the initial false negative result. This case highlights that clinicians should be aware of the postzone effect when using CSF or serum samples and consider requesting sample dilutions in patients with high pretest probability of invasive cryptococcal disease, even in the absence of a positive culture result.

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Ethical approval

No approval by an Institutional Review Board was required for this publication.

Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

CRediT authorship contribution statement

Chelsea Ware, Sherin Meledathu, Zoon Tariq, Rebecca Yee, Marc Siegel: writing, editing. John P. Lichtenberger, III: writing and image acquisition, editing of manuscript.

Declaration of Competing Interest

No conflict of interests to report.

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We acknowledge that all authors have contributed to this work and have seen and approved the manuscript. The contents of this manuscript have not been published and the manuscript is not being considered for publication elsewhere.

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