Clinical Report

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Overcoming Diagnostic Challenges in *Cryptococcal* Meningitis: Highlighting the Prozone Phenomenon

Superando los Desafíos Diagnósticos en la Meningitis por *Cryptococcus*: Destacando el Fenómeno de Prozona

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ABSTRACT

Cryptococcosis is a severe fungal disease associated with high mortality, especially in people with Advanced HIV Disease (AHD). Early diagnosis is critical for improving patient outcomes, and the use of rapid point-of-care test for *Cryptococcus* spp. antigen (CrAg) detection is crucial. However, diagnostic challenges remain, particularly the prozone effect, which can lead to false-negative results. We describe a case of prozone phenomenon in a patient with AHD diagnosed with cryptococcal meningitis. Initial CrAg testing in serum, as part of the clinical investigation process, yielded a negative result. However, after following a protocol for semi-quantification of CrAg, a positive result was observed at higher dilutions, confirming that the prozone effect was the cause of the false-negative result. Here we highlighted the importance of recognizing the prozone phenomenon in patients with high fungal burden and underscores the need for appropriate laboratory protocols to address this diagnostic limitation.

Key words: *Cryptococcus*, meningitis, HIV/AIDS, antigen, Prozone effect, False-negative results.

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RESUMEN

La criptococosis es una enfermedad fúngica grave asociada con una alta mortalidad, especialmente en personas con enfermedad avanzada por VIH (AHD, por sus siglas en inglés). El diagnóstico temprano es fundamental para mejorar el pronóstico de los pacientes, y el uso de pruebas rápidas en el punto de atención para la detección del antígeno de *Cryptococcus* spp. (CrAg) es crucial. Sin embargo, persisten desafíos diagnósticos, en particular el efecto de prozona, que puede generar resultados falsos negativos. Describimos un caso de fenómeno de prozona en un paciente con AHD diagnosticado con meningitis criptocócica. La prueba inicial de CrAg en suero, realizada como parte del proceso de investigación clínica, arrojó un resultado negativo. No obstante, tras aplicar un protocolo de semicuantificación del CrAg, se obtuvo un resultado positivo en diluciones más altas, confirmando que el efecto de prozona fue la causa del falso negativo. Este estudio resalta la importancia de reconocer el fenómeno de prozona en pacientes con alta carga fúngica y subraya la necesidad de contar con protocolos de laboratorio adecuados para abordar esta limitación diagnóstica.

Palabras clave: *Cryptococcus*, meningitis, HIV/AIDS, antígeno, efecto prozona, resultados falsos-negativos.

Introduction

Cryptococcosis, caused by yeast of *Cryptococcus neoformans* and *Cryptococcus gattii* species complex, is a globally reported disease associated with high mortality, particularly among immunocompromised individuals such as those with advanced HIV disease (AHD) [1]. It is estimated that *Cryptococcus* meningitis causes over 112,000 deaths annually, with the highest burden in sub-Saharan Africa [2]. In 2022, the World Health Organization (WHO) released its first fungal priority pathogens list, in which *C. neoformans* was identified as a top-priority fungal pathogen [3].

Early detection of cryptococcosis is crucial for improving patient outcomes, and the introduction of rapid point-of-care (POC) testing for the detection of *Cryptococcus* capsular antigen (CrAg) has played a significant role [1, 4]. Rapid CrAg detection offers several advantages over traditional diagnostic assays: faster turnaround times, high accuracy, and the ability to detect early infections in asymptomatic individuals. This allows for timely initiation of treatment, improving patient outcomes and reducing cryptococcosis-related deaths. Based on these advantages, the WHO, along with other international recommendations, advocates CrAg testing over other laboratory assays for the screening and diagnosis of cryptococcosis in individuals with AHD [5].

The detection of CrAg using lateral flow assays (LFA) has revolutionized point-of-care testing in resource-limited settings, enabling timely diagnosis and treatment initiation [1, 4]. However, despite the high reliability of CrAg tests, some diagnostic challenges remain, with the prozone effect being one of the most significant. The prozone phenomenon occurs when excessively high antigen concentrations in the specimen tested interfere with antigen-antibody binding, preventing the formation of detectable immune complexes. This paradoxical phenomenon leads to falsely negative or weakly positive results. While uncommon, the prozone effect is more likely to occur in immunosuppressed individuals, such as those with AHD, due to the high fungal burden. To mitigate this effect, strategies such as serial dilution of specimens are employed to reduce antigen concentration and ensure its accurate detection [6, 7]. Here we present a case report of a false-negative *Cryptococcus* antigen result in serum, caused by the prozone effect, in a patient with advanced HIV disease and cryptococcosis.

Clinical Report

A 44-year-old male patient diagnosed with HIV infection (Stage 3) nine years prior to the event (2014) was admitted to the emergency department at a Tertiary Care Center in Santiago, Chile, during January 2021. The patient had a history of non-adherence to antiretroviral therapy (ART). At the time of admission, he referred discontinuing ART for 4 years and resuming treatment one month ago. The patient was not receiving any antibiotic prophylaxis and the CD4 lymphocyte count, performed in July 2022, was 15 cells/mm³.

The patient reported a one-week history of holocranial headache, fatigue, and nausea. His companion also noted episodes of intermittent disorientation. On physical examination, the patient was alert and oriented to time and place, afebrile, and normotensive. No neurological deficits were observed, and meningeal signs were negative. He presented purpuric, elevated and painless skin lesions on both eyelids. Magnetic resonance imaging (MRI) demonstrated a hyperacute ischemic lesion. No nodular brain lesions were noted.

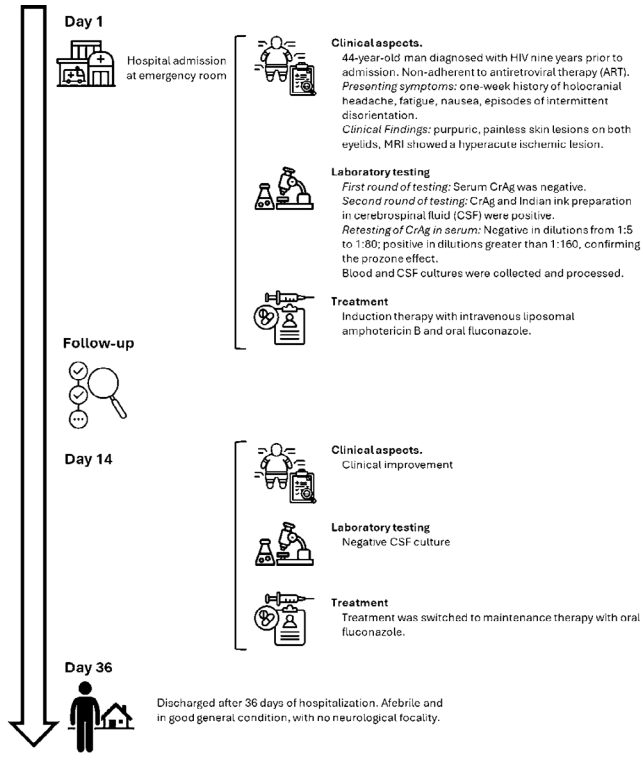
This scenario raised suspicion of an opportunistic infection, such as *Cryptococcus* meningitis. As an initial approach a serum CrAg test was requested in the emergency department, which was reported as negative (**Fig. 1**).

On the same day (Day 1), given the strong suspicion of *Cryptococcus* meningitis, a lumbar puncture was performed for cerebrospinal fluid (CSF) microbiological analysis. Indian ink preparation showed encapsulated yeasts compatible with *Cryptococcus* spp. and surprisingly a positive CrAg LFA was positive. These findings confirmed the diagnosis of cryptococcal meningitis. Induction therapy was initiated with intravenous liposomal amphotericin B and oral fluconazole. Cerebrospinal fluid (CSF) culture was reported as positive. The isolate recovered from culture was identified as *Cryptococcus neoformans* using MALDI-TOF mass spectrometry (VITEK-MS, BioMérieux, Marcy-l'Étoile, France) (**Fig. 1**).

Due to the discrepancy in CrAg results between serum and CSF specimens, a retest of the initial serum specimen was requested. This specimen was analyzed following the semi-quantitative procedure outlined by the product manufacturer (CrAg LFA, IMMY, Norman, OK, USA). The semi-quantitative testing revealed a false-negative result attributable to the prozone effect. False-negative results were observed in dilutions ranging from 1:5 to 1:80, while positive results were detected in dilutions from 1:160 to 1:2560 (the maximum dilution tested) (**Fig. 1**).

The patient demonstrated clinical improvement during induction therapy, and after 2 weeks of treatment initiation, the follow-up culture of the CSF was negative. Subsequently, the treatment was switched to maintenance therapy with oral fluconazole. The patient was discharged after 36 days, afebrile, in good general condition, and without neurological symptoms (**Fig. 1**).

Figure 1. Case timeline.



Discussion

In this report, we describe the challenges encountered during the diagnosis of a patient with cryptococcosis, initially misdiagnosed due to the prozone effect. We emphasize the importance of recognizing the prozone effect, particularly in patients at high risk for disseminated cryptococcosis. The correlation of clinical presentation and analysis of risk factors were fundamental for accurate diagnosis. Additionally, complementary CrAg testing of other specimens, such as cerebrospinal fluid (CSF), along with the use of rapid tests like Indian ink preparation, were essential for the prompt confirmation of cryptococcal disease and the involvement of the central nervous system in this patient.

Retesting of the serum using the semi-quantification procedure confirmed that the false-negative result was indeed due to the prozone effect, with positive results emerging only after serial dilutions were performed. However, this practice is not routinely conducted in most laboratories. Therefore, it is essential to utilize laboratory assays that can quickly

and effectively identify the prozone effect. A recently available alternative is a new lateral flow assay for the semi-quantification of *Cryptococcus* antigen (CrAg SQ LFA, IMMY, Norman, OK, USA). This semi-quantitative LFA provides rapid and accurate results, and it can estimate antigen loads in patients with *Cryptococcus* infections in a single test. One of its most significant advantages is its ability to eliminate false-negative results associated with the prozone effect [8, 9, 10].

Conclusion

This discrepancy between serum and CSF CrAg results underscores the importance of considering the prozone effect in patients with high fungal burdens, as delayed diagnosis could have severe consequences. In conclusion, this case highlights the diagnostic complexities of *Cryptococcus* meningitis in immunocompromised patients, particularly those with advanced HIV. Recognizing and addressing diagnostic challenges, such as the prozone effect, are critical for timely diagnosis and management, ultimately leading to improved patient outcomes.

Conflict of interest statement

The authors declare no conflicts of interest. Diego H. Caceres has been an IMMY employee since December 2021.

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Ethical responsibilities

This study was conducted in accordance with ethical standards and applicable regulations.

Author Contributions

Conceptualization, CEV and DHC; methodology, CEV and DHC; formal analysis, CEV and DHC; investigation, CEV and DHC; data curation, CEV and DHC; writing—original draft preparation, CEV and DHC; writing—review and editing, CEV and DHC.

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