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False-negative result of serum cryptococcal antigen lateral flow assay in an HIV-infected patient with culture-proven cryptococcaemia

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ABSTRACT

The detection of cryptococcal capsular antigen (CrAg) is very sensitive and specific, however false-negative results have been reported, mostly in cerebrospinal fluid. We report the case of an HIV-infected patient with CD4 = 42 cells/mL, asthenic, negative serum CrAg lateral flow assay (LFA) and culture-proven cryptococcaemia. Despite the high accuracy of LFA, false-negative result is possible. Careful clinical evaluation and close follow-up are relevant.

1. Introduction

Cryptococcosis is a severe opportunistic fungal infection with high morbidity and mortality, caused predominantly by species of *Cryptococcus* complexes (*C. neoformans* and *C. gatti*) [1]. This fungus particularly affects people living with HIV/AIDS (PLHAs) with advanced disease (CD4 count < 100 cells/mL). The presentations of cryptococcosis is broad, ranging from respiratory tract colonization to cryptococcaemia and cryptococcal meningitis [2].

The cryptococcal antigen (CrAg) lateral flow assay (LFA) (Immuno-Mycologics, OK, USA) is a key advance in the diagnosis of cryptococcosis in PLHAs. The LFA is in excellent agreement with cultures, latex agglutination and immuno enzymatic assay (ELISA) in serum and cerebrospinal fluid (CSF) [3]. It has 100% (97.6%–100%) serum sensitivity, 96.8% (93.7%–98.6%) specificity and 100% (98.1%–100%) negative predictive value compared with culture and latex agglutination [3,4]. Serum lateral flow assay had a pooled sensitivity of 100% when compared with blood culture and of 95.6% when compared to serum enzyme immunoassay [5]. Cryptococcosis with a false-negative antigenic test has been rarely reported in the literature [6–8]. The CrAg LFA has some advantages over conventional diagnostic methods: results are available in less than 10 minutes, little training is required for use and interpretation, minimal laboratory infrastructure is required and refrigerated storage is not necessary [4].

This case report presents an episode of false-negative cryptococcal antigenemia by LFA, demonstrated by a positive blood culture for *Cryptococcus* spp. of a matched sample, highlighting the need for knowledge of this situation and discussion about its possible causes.

2. Case

A 57-year-old man from Goiânia (State of Goiás, Brazil) was diagnosed with HIV thirteen years before, without any follow-up since then. He had a recent hospitalization for consumptive syndrome, chronic diarrhea and liver microabscesses (T0). On that occasion, he received antibacterial systemic treatment (ceftriaxone 2g/day + metronidazole 1,5g/day for 14 days), antiparasitic treatment (nitazoxanide 1g/day for 3 days), was started on antiretroviral therapy (ART; tenofovir disoproxil fumarate + lamivudine + dolutegravir) and primary prophylaxis with sulfamethoxazole-trimethoprim (800mg/160mg/day) and azithromycin (1,5g/week).

At the time of hospital stay (Day 0), his test results were as follows: lymphocytes T $CD4^+$ (LT $CD4^+$) count of 42 cells/mL (5%); HIV viral load (VL), 805,439 copies/mL (log 5.9); negative cryptococcal antigen

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by latex agglutination (Immuno-Mycologics, OK, USA); and negative bacterial and fungal blood culture. More than one month later (T1, Day +47), during outpatient follow-up he complained solely of asthenia, without other systemic or neurological symptoms. A blood sample was collected for LFA cryptococcal antigenemia, blood culture and biochemical examinations as part of a study recruitment [9]. The serum CrAg LFA was negative, and the patient was advised to maintain clinical follow-up and adherence to ART.

However, the patient was lost to follow-up and we received the CrAg-matched blood culture with growth of beige mucoid yeast colonies with positive Indian ink suggestive of *Cryptococcus* spp. At this time, the reference laboratory was not performing differentiation between *Cryptococcus* complex species (*C. neoformas* and *C. gatti),* however, environmental species were rule out by biochemical tests. A new outpatient visit (T2, Day +173) was performed only after 4 months of T1. Between T1 and T2, the patient presented progressive improvement, had a 30 kg weight gain, was on regular use of medications and received no systemic antifungal therapy.

At T2 (Day +173), the source of cryptococcaemia was investigated. The serum CrAg tests performed were positive by LFA (Fig. 1 A) and by latex agglutination (LA, titration 1:64). Blood and urine cultures were negative for fungi. His recent LT CD4$^+$ was 223 cells/mL (11%) and VL was undetectable. His general laboratory results were substantially improved. Table 1 describes the results of laboratory exams performed during this time.

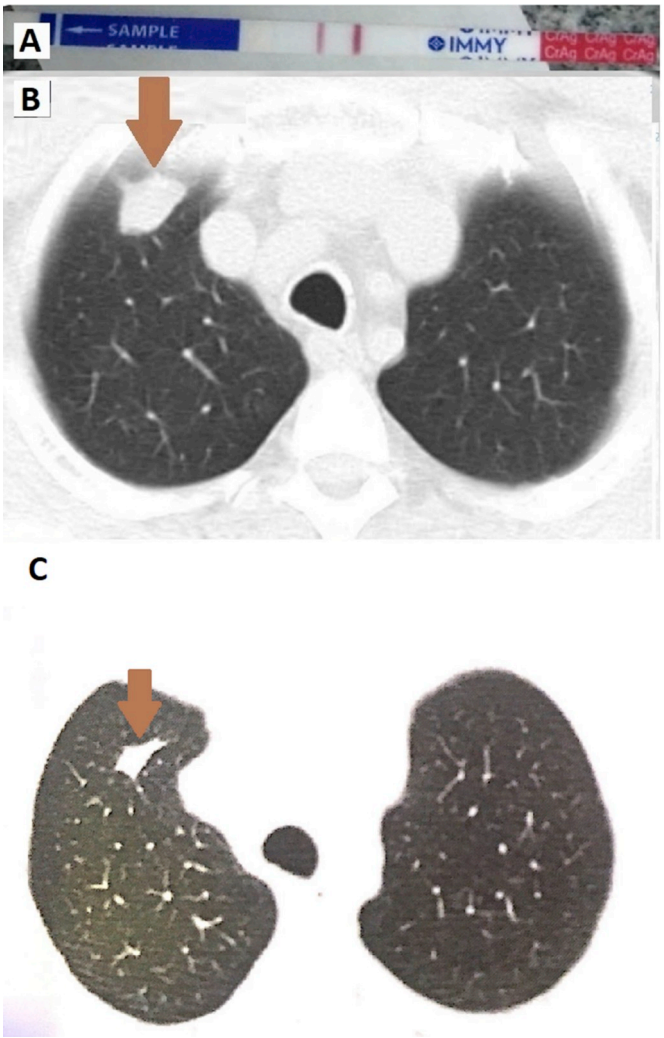

Fig. 1. A) Lateral flow assay test showing the positive reaction (Day +173). **B)** Chest computed tomography (CCT) scan: a nodule of 2.2 × 1.2 cm in the upper lobe of the right lung (Day +173). **C)** Control CCT scan: nodular atelectatic opacity in the right upper lobe of healing aspect (Day + 502).

Table 1
Laboratory exams results.

Exams	Day 0[a]	Day +47[b]	Day +173[c]
TL CD4$^+$ count (cells/mL)	42 (5%)	-	223 (11%)
Viral load (copies/mL)	805,439 (5.9)	-	Undetectable
CrAg Latex agglutination - IMMY[d]	Negative	-	Positive 1:64
CrAg LFA - IMMY[d]	-	**Negative**	Positive
Blood Culture	Negative	*Cryptococcus* spp.	Negative
Haemoglobin (g/dL)	-	11	16.3
Haematocrit (%)	-	35	48
White blood cell (cells/mm^3)	-	4590	3630
Platelets (cells/L)	-	233×10^9	151×10^9
Albumin (g/dL)	-	2.79	3.72
CrAg LFA (CSF) - IMMY[d]	-	-	Negative

[a] Time zero: first hospitalization, day 0.
[b] Time 1: first outpatient consultation, day +47.
[c] Time 2: second outpatient consultation, day +173.
[d] Immuno-Mycologics, OK, USA.

Cryptococcal meningitis was ruled out by lumbar puncture and CSF analysis. A computed tomography (CT) scan of the chest revealed a single nodule with soft-tissue density and regular contour in the anterior segment of the upper lobe of the right lung, measuring 2.2 × 1.2 cm (Fig. 1 B). He was treated for pulmonary cryptococcosis with fluconazole 800 mg for 2 weeks, followed by fluconazole 400 mg for a total of 6 months (from Day +173 to Day +356). The patient maintained follow-up, adherence to ART, without any symptoms suggestive of cryptococcosis after the end of fluconazole treatment (Day +356).

During late follow up, the patient was asymptomatic and brought a control thoracic CT scan (Day +502) showing a nodular atelectatic opacity in the right upper lobe of healing aspect (Fig. 1 C). At this moment, 15 months after the introduction of fluconazole therapy (Day +669), a new serum CrAg LFA was performed and the result was negative.

3. Discussion

Here, we report an unusual case of an HIV-infected patient with cryptococcaemia and a negative CrAg LFA in a paired sample of serum. In general, a false-negative CrAg test may be the result of: low fungal load; prozone reaction due to high antigen titers; presence of immunocomplexes preventing release of glucuronoxilomanan-antigen; samples transported in inappropriate vials; or hypocapsular or acapsular strains of *Cryptococcus* spp [6]. Human infections caused by acapsular *Cryptococcus* or poorly encapsulated strains can be identified only after tissue biopsy or tissue culture [8,10,11].

In cases where there is a very high antigenic concentration, the antigen-antibody reaction may not occur and the test may present as false-negative, the so-called prozone (“Hook”) effect or postzone effect [7,12]. Most reports refer to false-negative tests on cerebrospinal fluid (CSF) [6,8,13], with limited data resembling serum samples [11].

It is reported an increase in LFA sensitivity for the diagnosis of cryptococcal meningitis when CSF samples suspected of having a high organism load is diluted before the assay is performed [11,12]. One study described a patient from Malawi who had a positive India ink stain in CSF, with a negative initial CrAg LFA test. They reached positivity after semi-quantitative analysis on the CSF, demonstrating different results at progressive titers: 1:5, negative; 1:10, weakly positive; 1:20, positive; 1:40, weakly positive; and 1:80, negative [7].

Another case report described a patient with slow clinical progression of untreated cryptococcosis from leptomeningitis to a parenchymal form of the disease, with all negative CrAg LFA tests. It was explained by a capsule-deficient strain of *Cryptococcus* with reduced virulence, delaying the right diagnosis [6].

In the present case, an HIV-infected patient with advanced disease presented cryptococcaemia with a concurrent negative serum CrAg LFA. Interestingly, he had only unspecific complaints and showed good outcome, despite not receiving antifungal therapy. Cryptococcaemia is classically associated with increased odds of mortality (odds ratio, 5.09; 95% confidence interval, 2.54–10) in retrospective studies [14]. The cause of spontaneous and unexpected resolution of cryptococcaemia is unknown, but it is possible that a transient subclinical episode may have occurred. Subclinical or asymptomatic cryptococcal meningitis is well described and theoretically subclinical crypococcaemia can be possible in a subset of cases as suggested with temporal sequence of CrAg concentration in blood during progression of disease from pulmonary infection to meningitis [15,16].

The regular use of ART and effective immune restoration may have contributed to the benign outcome. In addition, the concomitant negative serum CrAg LFA may be explained due to; (i) more probably, a prozone effect; or (ii) either acapsular or hypocapsular specimens [6–8]. Unfortunately, we no longer have the culture specimen stored or possibility of titration of CrAg LFA to evaluate these hypotheses. In addition, the positivity of CrAg LFA and LA performed at T2 (Day +173) refutes this second hypothesis. On the other hand, postulating the prozone effect starts from the principle of assuming a high fungal load, a fact that is intriguing considering the oligosymptomatic clinical picture and the benign evolution of the case.

In the present case, an asymptomatic and isolated pulmonary nodule, probably a cryptococcoma, was observed during follow up (Day +173). It is possible that this lesion was present in the first admission (T0) and could be the source of the transient cryptococcaemia. The significant improvement shown in control CT (Day + 502) and the negativity of antigen testing (Day +669) reinforce this hypothesis.

Despite the high accuracy of the antigenic test by LFA, careful clinical evaluation, culture assessment, and close follow-up are relevant. Better tracking of patients who have been screened for CrAg is needed, because false-negative cases may occur and should be considered in immunocompromised patients.

Declaration of competing interest

JEV has received fees for lectures from Gilead and United Medical and has received donation of CrAg LFA kits for clinical studies from IMMY, both unrelated to this study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mmcr.2019.10.009.

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