Cryptococcus neoformans meningitis with negative cryptococcal antigen: Evaluation of a new immunochromatographic detection assay

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Abstract

Detection of cryptococcal antigen in serum or cerebrospinal fluid allows cryptococcal meningitis diagnosis within few hours with >90% sensitivity. In an HIV-positive patient with *Cryptococcus neoformans* meningitis, initial antigen detection by immunoagglutination was negative. We thus evaluated a new immunochromatographic detection assay that exhibited a higher sensitivity.

Keywords: Antigen test, *Cryptococcus neoformans*, HIV, immunochromatography, meningitis

Original Submission: 14 August 2014; **Revised Submission:** 10 November 2014; **Accepted:** 8 December 2014

Available online 19 December 2014

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Cryptococcus neoformans has emerged as an important cause of pneumonia and meningoencephalitis among patients with reduced cell mediated immunity. Among patients infected with human immunodeficiency virus (HIV), most of the cases of cryptococcosis occur with a CD4 cell count of <100 cells/mm^3 [1]. Diagnosis is based on blood or cerebrospinal fluid (CSF) cultures and on immunoassays often used to detect *C. neoformans* surface capsular polysaccharide glucuronoxylomannan (GXM) shed during infection [2–5]. In HIV-infected patients, sensitivity of cryptococcal antigen detection in CSF approaches 99% [6]. Nevertheless, cryptococcosis with false-negative antigen results has been reported [7–9].

In the present study, we report a case of cryptococcal meningoencephalitis in an HIV-infected patient with initial negative cryptococcal antigen detection. We reviewed cryptococcal antigen assays at our institution over the last 25 years and evaluated a new immunochromatographic detection assay that exhibited a higher sensitivity.

In July 2013, a 29-year-old woman was seen at our outpatient infectious diseases clinic for an HIV infection that was diagnosed 4 months before in Cameroon. Antituberculosis treatment for a likely pulmonary tuberculosis had been started at HIV diagnosis. The patient complained about fever and cough that had lasted for several weeks. At baseline workup, CD4$^+$ cell count was 140/mm^3, and HIV virus load was 1.2 × 10^6 copies/mL. A first serum cryptococcal antigen (July 2013) was negative using the Pastorex immunoagglutination assay (Bio-Rad, Marnes la Coquette, France) (Fig. 1). A right lower lobe infiltrate was seen on chest x-ray. Bronchoalveolar lavage was negative for *Mycobacterium tuberculosis* (PCR, culture) and fungi (silver stain, culture). Cotrimoxazole for *Pneumocystis jirovecii* pneumonia prophylaxis was started (while waiting for the result of HIV genotypic analysis to initiate antiretroviral therapy), and antituberculosis treatment was pursued. Two weeks later, fever persisted and the patient developed diffuse intense holocranial headache that led to her admission to the emergency department. At examination, her temperature was 38.2°C, and her vital signs were normal. There was no neck stiffness, and the neurologic examination was normal. Brain magnetic resonance imaging showed a contrast-enhancing nodule about 6 mm of diameter in the left posterior parietal lobe without edema or signs of intracranial hypertension. CSF opening pressure was 7 cm H_2O, and CSF examination revealed 192 leucocytes/mm^3 (lymphocytes 88%), while protein level was 1775 mg/L, lactate level was 2.8 mmol/L and CSF/blood glucose ratio was 0.4. No microorganisms were observed on CSF staining. Bacterial and mycobacterial cultures were negative. Cryptococcal antigen in serum and CSF was negative (Fig. 1). Two days later, the CSF culture became positive. Microscopic observation revealed the presence of yeastlike organisms that we identified as *C. neoformans* using MALDI-TOF (matrix-assisted laser desorption-ionization time-of-flight) analysis (Bruker Daltonics, Leipzig, Germany) with a spectral score above 2

http://dx.doi.org/10.1016/j.nmni.2014.12.003

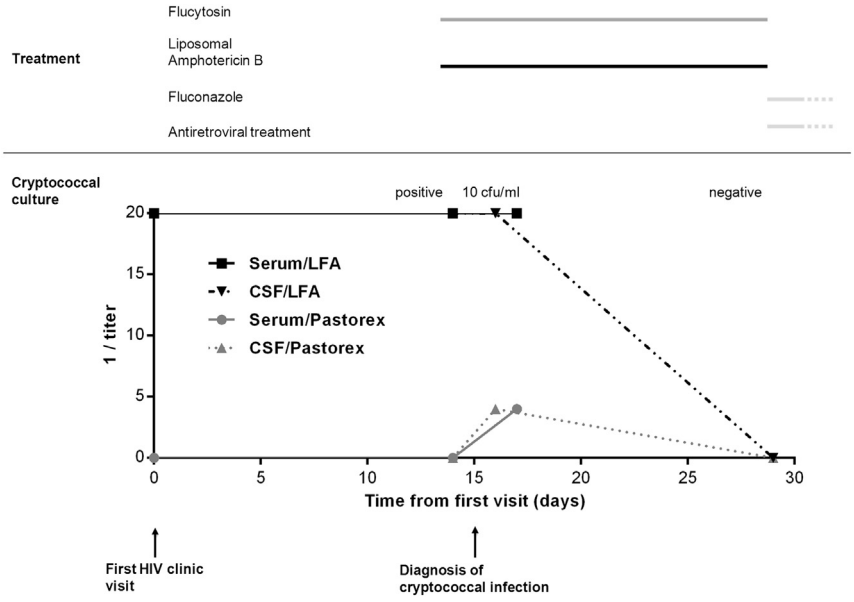

FIG. 1. Time course of the case study and treatment. The buttom part of the figure shows results of cryptococcal antigen titers and cryptococcal culture. Cryptococcal antigen titers were determined by immunoagglutination (Pastorex) and lateral flow assay (Immunomycologics Inc., IMMY). Quantitative culture was achieved as follows: four drops of 100 µL, 50 µL, and 10 µL were deposited in duplicate on a brain–heart infusion plate supplemented with blood and incubated at 37°C with CO_2. Antifungal and antiretroviral treatment is depicted in the upper part of the figure. Antifungal treatment consisted of liposomal amphotericin B (5 mg/kg) and intravenous flucytosine (25 mg/kg every other day). After 2 weeks of combined therapy, the patient was apyretic and headache disappeared; lumbar puncture revealed normal opening pressure and decreased amount of protein (862 mg/L), and CSF culture was sterile. Cryptococcal antigen detection in the CSF became negative as well. Antifungal treatment was switched to fluconazole (400 mg once daily) and antiretroviral treatment was initiated, without relapse of cryptococcal meningitis.

[10–12]. The same day, a CSF was collected, which came positive for GXM antigen (titer 1:4) using the Pastorex immunoagglutination assay (Fig. 1). We initiated antifungal therapy with liposomal amphotericin B 5 mg/kg iv once daily and flucytosine 25 mg/kg every 6 hours during the first 2 weeks, followed by fluconazole 400 mg by mouth once daily according to current guidelines [13], with a good outcome. Antiretroviral therapy with tenofovir, emtricitabine, raltegravir, darunavir and ritonavir was started after 2 weeks of antifungal therapy.

In our 1027-bed tertiary-care university hospital, we use the immunoagglutination assay Pastorex Crypto Plus 61747 (Bio-Rad), based on latex beads coated with anti-GXM mouth monoclonal antibodies [14,15]. From 1996 to 2013, 1759 samples (sera and CSF) from patients with suspicion of *C. neoformans* infection were analysed using this assay; among them, 152 samples tested positive (Fig. 2). The present case is the first of meningitis with positive *C. neoformans* culture and negative cryptococcal antigen agglutination in our hospital. A negative agglutination due to a prozone effect that can occur in high antigen titers has been excluded by serial dilution and by retesting of the sample. We thus hypothesize that the negative result is due to a low fungus load (10 *C. neoformans* cfu/mL at quantitative culture of the initial sample), possibly explained by partially preserved immunity (CD4 cells above 100/mm^3).

Because sensitivity may vary among different commercial immunoassays, we retrospectively tested the negative samples of our patient with the recent lateral flow assay (LFA) detection system from Immunomycologics Inc. (IMMY) [6,16]. This immunochromatographic Point-of-Care test is based on the qualitative and semiquantitative detection of GXM in sera and CSF. The experimental sensitivity of the IMMY-LFA system (C5 to C95 interval = 1.0–1.5 ng/mL) determined with purified GXM is higher than the sensitivity of the Pastorex immunoagglutination system from Bio-Rad (detection limit 50 ng/mL) [17]. To validate the LFA assay in our laboratory, we retrospectively tested 50 CSF and sera from the collection of our hospital (30 positive and 20 negative samples) previously analysed with the Pastorex system. We found 100% agreement between the two systems. Antigen titers measured for positive

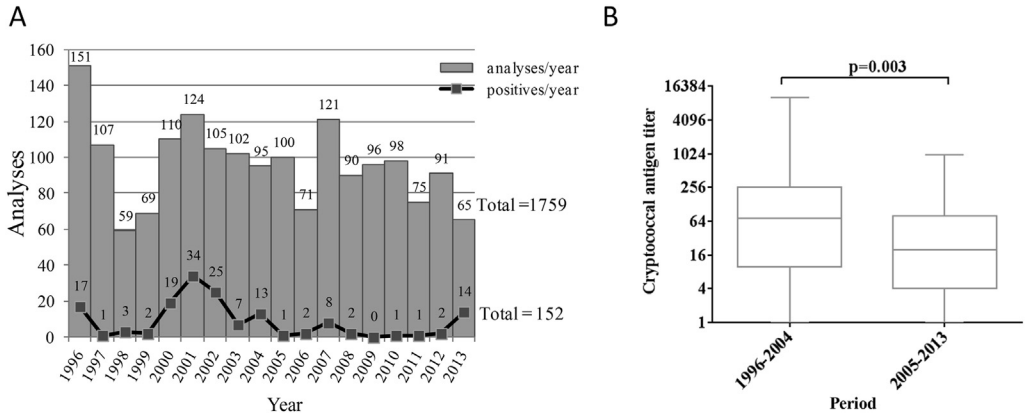

FIG. 2. *Cryptococcus neoformans* infection over 18 years. (A) Total number of cryptococcal antigen detection by immunoagglutination and number of positive results in our hospital from 1996 to 2014. Results were obtained with the Bio-Rad assay Pastorex Crypto Plus 61747. (B) Evolution of cryptococcal antigen titer among positive results: comparison between 1996–2004 and 2005–2013 (comparison by Mann-Whitney test). Antigen titer for positive samples was determined by serial dilution according to the manufacturer's procedure.

samples were always higher with the IMMY-LFA system than with the Pastorex assay (Fig. 3; Supplementary Table 1). The samples from our patient that were negative with the Pastorex immunoagglutination assay came positive with the IMMY-LFA system (titer 1:20) (Fig. 1). This suggests a higher sensitivity of the IMMY-LFA compared to immunoagglutination assays. This is consistent with two recent large studies, performed on 421 sera from HIV patients in Colombia and 589 sera and 411 CSF in the United States, respectively [18,19]. The negative case that we report in this article might be due to the low fungus load of *C. neoformans* (10 cfu/mL), which might be close to the detection limit (Fig. 1).

The incidence of cryptococcal meningitis has decreased since the advent of effective antiretroviral therapy [20,21]. Similarly, infections with high antigen titer are less likely to occur. In our hospital, we observed decreasing antigen titer for positive samples over the last 18 years (Fig. 2B), with a median titer of 72 during 1996–2004 versus 20 during 2005–2013 (p 0.003, comparison by Mann-Whitney test). Although we did not have comprehensive data on quantitative cultures, this suggests infections with a lower fungus load. In this context, cryptococcosis cases with negative antigen results are more likely to occur. Other causes of false-negative antigen results can be high antigen titers (>1:256) leading to a prozone reaction [22,23] or the presence of immune complexes that prevent the shedding of GXM antigen [24]; alternatively, low antigen release could be associated with poorly encapsulated strains [25–27].

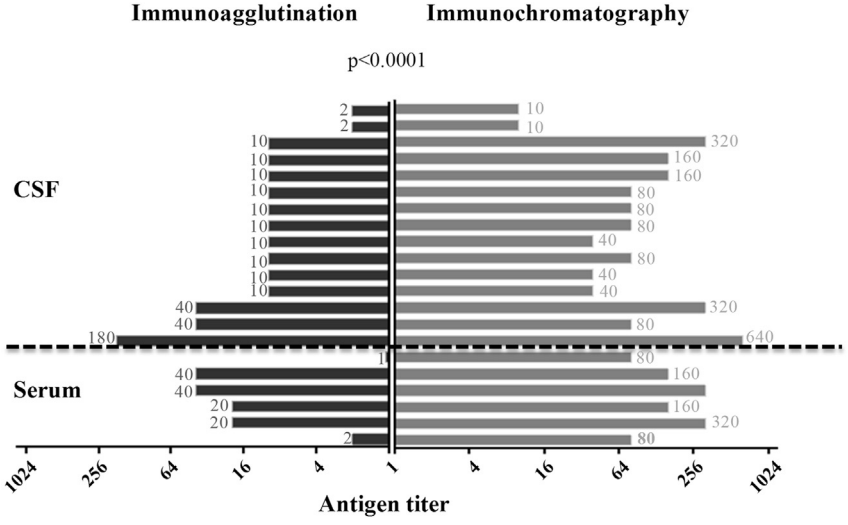

FIG. 3. Evaluation of the lateral flow assay (LFA) detection system from Immunomycologics Inc. (IMMY). A total of 50 samples (30 positive and 20 negative) previously analysed with the immunoagglutination assay Pastorex Crypto Plus 61747 (Bio-Rad) were retrospectively tested with the IMMY-LFA (Supplementary Table 1). The 20 samples that were negative with the Pastorex assay also came negative with the LFA assay (data not presented). In 9 cases, immunochromatography was performed but the titration could not be done because the volume of stored CSF was not sufficient. Antigen titers were always higher with the IMMY-LFA system than with the Pastorex assay (comparison by Wilcoxon signed rank test).

In conclusion, negative antigen results do not permit the exclusion of cryptococcal infection, especially in the setting of low fungus load. Immunochromatography systems should be preferred over immunoagglutination assay, as they are easier to handle and more sensitive.

Conflict of interest

None declared.

Acknowledgements

We are grateful to all of the technicians of the Diagnostic Microbiology Laboratory of the University Hospital of Lausanne for their technical contribution. In particular, we thank Maria Senra-Ortiz and Christian Durussel.

Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.nmni.2014.12.003.

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