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Rapid diagnosis of cryptococcosis using an antigen detection immunochromatographic test

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Summary *Objectives:* Current methods for cryptococcal antigen detection have some limitations. This study aimed at evaluating a lateral flow assay (LFA) for the diagnosis of cryptococcosis in a French University medical center.
Methods: A retrospective study was performed on samples collected from patients with a definitive diagnosis of cryptococcosis (group I 66 samples; 28 patients) or with non-*Cryptococcus* invasive fungal infection (group II 18 samples; 17 patients). In addition, 274 samples from 205 consecutive patients, either suspected of cryptococcal infection or routinely

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screened during their follow-up, were prospectively tested (group III). Cryptococcal antigen was assayed using LFA and an EIA. A latex-based test was used for confirmation.
Results: Sensitivity calculated on group I and specificity on group II, were respectively at 100% and 90.0%. Two false positives were related to *Trichosporon* fungemia. Per-sample analysis on group III revealed sensitivity, specificity, positive and negative predictive values all at 100% for CSF, and at 100%, 98.9%, 75% and 100%, respectively for serum samples. LFA enabled the diagnosis of two cases of asymptomatic cryptococcosis.
Conclusion: The excellent diagnostic value and practicality (visual reading results in 15 min) of LFA make it fully appropriate for the diagnosis of cryptococcosis in this particular setting.

Introduction

Cryptococcosis is caused by basidiomycetous yeasts of the *Cryptococcus neoformans/Cryptococcus gattii* species complex. In most cases, cryptococcosis is responsible for life-threatening meningitis complicating the course of various states of immunosuppression such as HIV infection, unrevealed or uncontrolled by antiretroviral therapy, solid organ transplantation, chronic lymphoid leukemia, liver fibrosis, sarcoidosis, or any kind of affection requiring long-term corticosteroid therapy.[1] While the incidence of cryptococcosis has declined with the advent of Highly Active Anti-Retroviral Therapy (HAART), its outcome still remains poor, even in developed countries, with a mortality rate calculated in a large French survey at 17%, 3 months after the diagnosis.[2] As is the case for the majority of invasive fungal infections, the prognosis of cryptococcosis depends on an early diagnosis. The diagnosis of cryptococcosis combines direct diagnosis tests to visualize (Indian ink staining) and isolate (culture on appropriate medium) the pathogen from different fluids, and serodiagnostic assays (antigenic detection), mainly in serum and CSF. However, direct diagnosis can lack sensitivity in case of low fungal inoculum. Moreover, cultures supply a delayed result, since they usually take more than 72 h to be positive.[3] The detection of the capsular glucuronoxylomannan (GXM) antigen is thus an important complementary diagnostic tool. Antigen can be detected using immunoenzymatic (EIA) methods or agglutination of sensitized latex particles (LA) with good performances. However, there are some limitations of these tests that render them not fully suitable for rapid diagnosis, notably during night duty. Indeed, a preliminary 15-min centrifugation is mandatory for both EIA and LA tests. An additional time of 45 min (incubation of sample, enzyme conjugate, substrate and finally stop solution) is required to obtain results with EIA. For the latex-based test, an incubation step of the samples with pronase may be required in order to eliminate immune complexes, and reach an appropriate level of sensitivity.[4] Moreover, the test may be difficult to read, notably in the case of weak agglutination.

Recently, a new lateral flow assay (LFA) has been evaluated positively for the diagnosis of AIDS-related cryptococcal meningitis, mainly in resource-limited settings.[3,5,6] The purpose of this work was to evaluate this commercial test for the diagnosis of cryptococcosis in a French University hospital.

Material and methods

Samples

Samples were divided into three groups according to the patient's condition. Group I corresponded to 66 samples previously collected from 28 patients with a proven diagnosis of cryptococcosis: they either had positives cultures for *C. neoformans/C. gattii*, or two different, positive antigen detection assays, or a positive Indian ink staining plus detection of the antigen. All patients but two, were HIV-infected. There were 24 sera, 30 CSF, 7 urine samples and 5 BAL fluids. Group II gathered 18 samples (1 CSF and 17 sera) from 17 patients with proven or probable invasive fungal infection other than cryptococcosis. Those infections were due to *Trichosporon asahii* (2 patients; 2 sera, one CSF), *Histoplasma capsulatum* (2 patients; 2 sera), *Aspergillus fumigatus* (4 patients; 4 sera), *Pneumocystis jirovecii* (3 patients; 3 sera), *Candida albicans* (3 patients; 3 sera), *Rhodotorula* sp (3 patients; 3 sera). In addition, one serum and one BAL fluid, which results were considered as false positives, were included. They were collected from two patients for whom the diagnosis of cryptococcosis was finally ruled out since antigenic detection occurred only once and only with an Enzyme Immunoassay test.

Finally, group III corresponded to 90 CSF and 184 sera included prospectively and collected from 205 consecutive patients, who were either suspected of cryptococcosis (neurological symptoms) or screened systematically (routine follow-up of HIV-positive patients) for cryptococcal antigen. In the case of clinical symptoms or positive antigen detection, these patients were investigated in depth, mostly using imaging, blood-cultures, lumbar puncture, and broncho-alveolar lavage (BAL) subjected to Indian ink staining and culture.

Antigen detection

Antigen detection was performed using the EIA Cryptococcal antigen test (Premier Cryptococcal Antigen, Meridian, Bioscience, France) and a latex assay (Crypto-Ag LA, Fumouze, France) according to the manufacturers' recommendations. Both tests use 50-μl specimens per reaction. For the EIA test, reading was done using a spectrometric dual wavelength (450/630 nm). Optical density $\geq$0.1 was considered as positive, $<$0.07 as negative and indeterminate between both. For the latex-based test, serum (not