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Assessment of an Antigen Lateral Flow Assay (LFA) For Point of Care Diagnosis of Cryptococcosis in Senegal

Sow D[1]*, Sylla K[2], Ndiaye M[2], Lelo S[2], Tine RC[2], Badiane AS[2] and DiengT[2]

[1]Department of Parasitology-Mycology, UFR Health Sciences, University of Gaston Berger, Saint-Louis, Senegal

[2]Department of Parasitology-Mycology, University of Cheikh Anta Diop, Senegal

***Corresponding author:** Doudou SOW, Department of Parasitology-Mycology, University of Gaston Berger, Saint-Louis, Senegal, Tel: 775425028 (221); Email : doudou.sow@ugb.edu.sn

Research Article

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Abstract

Background: Cryptococcal meningitis is a medical emergency. Therefore, simple, rapid and efficient diagnostic techniques are needed. New rapid diagnostic tests based on the detection of cryptococcal antigen have been developed. The aim of our study was to evaluate the performance of the Dynamiker Cryptococcal Antigen Lateral Flow Assay (LFA) in our routine diagnosis activities.

Materials and Methods: We performed a prospective study from November 2017 to August 2018 at the Parasitology-Mycology laboratory of the CHNU of Fann in Dakar. Biological samples collected from HIV-infected patients hospitalized at the infectious diseases department of CHUN de Fann were used. Serum, CSF and urine samples received in the laboratory were examined using mycological techniques and the cryptococcal latex agglutination test (CLAT). The performance of the Dynamiker test was evaluated considering CLAT as the reference method.

Results: A total of 39 samples were examined: 14 CSF, 24 sera and 1 urine sample. The average age was 44.5 years with a predominance of female patients (64.1%). The mean CD4 count was 73 cells/mm^3. Among the 39 samples tested, 23.1% were CLAT positive while 30.8% were LFA positive. The sensitivity and specificity rates were 100% and 75% respectively. The percentage of correlation between the two methods was 92.3%. The intensity of the bands on the LFA was high en 66.7% of cases.

Conclusion: The excellent sensitivity of the rapid Cryptococcal Antigen Lateral Flow Assay and the rapidity of obtaining results (less than 15 minutes) make it suitable for routine diagnosis of cryptococcal meningitis.

Keywords: Cryptococcal Meningitis; Latex Agglutination; Lateral Flow Assay

Introduction

Cryptococcus neoformans is encapsulated yeast that is responsible for life-threatening infections, particularly in immunocompromised patients [1] cryptococcal meningitis (CM) is responsible of a high mortality rate reaching 600,000 deaths per year particularly in resource-limited countries [2]. Cryptococcal meningitis also occurs in patients with other forms of immunosuppression and in apparently immunocompetent individuals [3,4]. In Sub-Saharan Africa countries, there are more than 500,000 deaths each year due to CM, which may exceed those attributed to tuberculosis [5]. Cryptococcal meningitis is also the leading cause of community-acquired meningitis in parts of sub-Saharan Africa where the HIV prevalence is high, ahead of *Streptococcus pneumoniae* and *Neisseria meningitidis* [6,7]. Mortality from HIV-associated CM remains high (10–30 %), even in developed countries, because of the inadequacy

of current antifungal drugs and combinations, and the complication of raised intracranial pressure [8,9]. In the developing world, patients tend to present several weeks and months later [10-12].

Key factors influencing survival are the fungal burden at presentation and the rate of sterilization of cerebrospinal fluid (CSF) with combination treatment. The rapid and accurate laboratory diagnosis of CM is important to enable the timely use of appropriate treatment and prevent diagnostic delays contributing to increased CSF fungal loads and poor clinical outcomes. The standard diagnostic methods include India ink staining, the conventional cryptococcal latex agglutination test (CLAT), and culture of CSF which is generally performed by trained technical staff, predominately at centralized laboratories. The CLAT is labor intensive, and sample batching may further delay the turnaround time [13,14]. Cultures may be negative or slow to grow for patients with low fungal burdens or those already receiving treatment. Prolonged fungal culture often results in bacterial contamination and further delays as the isolate is purified. Given the high mortality rate of CM, it is clear that initiation of treatment cannot be delayed pending culture results.

The laboratories Dynamiker Biotechnology (Tianjin) Co., Ltd. have recently developped a cryptococcal antigen lateral flow assay (LFA), a commercially available rapid immunochromatographic diagnostic test for the detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in human serum and cerebral spinal fluid [15]. The test uses lateral flow technique and double antibody sandwich format. The test uses specimen wicking to capture gold-conjugated, anti-cryptococcal antigen (anti-CrAg) monoclonal antibodies and gold- conjugated control antibodies on the test membrane. The LFA is cost-effective, stable at room temperature, and easy to perform. Early demonstration studies on the performance of the test have shown that clinical sensitivity and specificity is 98.68% and 98%, respectively (Manufacturers). The aim of this study was to evaluate the accuracy and the reliability of the Dynamiker Cryptococcal Antigen Lateral Flow Assay (LFA) in routine diagnosis compared to india ink staining, CLAT and culture in an african setting.

The specific objectives was to describe the percentage of positive and negative results by the Dynamiker Cryptococcal Antigen Lateral Flow Assay (LFA) in the diagnosis of cryptococcal meningitis in Fann Hospital compared to india ink staining, CLAT and culture, to determine the sensitivity, specificity of Dynamiker Cryptococcal Antigen Lateral Flow Assay (LFA) and to assess the level of agreement between the Dynamiker Cryptococcal Antigen Lateral Flow Assay (LFA) and the standard diagnosis methods.

Materials and Methods

Study Sites

The study was conducted in the department of Parasitology-Mycology in Fann Teaching Hospital in Dakar from October 2017 to March 2018. Cerebrospinal fluid and serum samples were collected in patients with suspected Cryptococcal meningitis hospitalized in the Infectious Diseases Clinic in Fann Teaching Hospital. Patients included those with clinically suspected or confirmed HIV infection with signs and/or symptoms suggestive of meningitis.

Study Design

This is a prospective study to assess the test in 40 patients with suspected or confirmed cryptococcosis. The study population included 30 positive cases (20 CSF specimens and 10 serum samples) diagnosed with standards methods (India ink staining, culture and/or CLAT) and 10 negative controls.

- In serum samples: positive results with CLAT was considered as positive cases
- In CSF: positive results from India ink staining and/or culture and/or CLAT was considered as positives cases.

Laboratory Investigations

The Dynamiker Cryptococcal Antigen Lateral Flow Assay was performed on CSF and serum samples according to the manufacturer's instructions : 40 µl of buffer was mixed with 40 µl of CSF or serum in a disposable tube, followed by insertion of the LFA test strip. Results was obtained after 10 min. A positive result was reported if two visible lines developed over the control and test areas, and a negative result was reported if a single control line is present.

The standards techniques including india ink staining, fungal culture and CLAT were performed on CSF samples according to the laboratory protocols. Cerebrospinal fluid specimens for culture of *C. neoformans* was centrifuged and the sediment. Inoculated onto Sabouraud's dextrose agar and incubated at 37 °C for 14 days. The suspected colonies were identified by microscopic examination and its ability to produce urease on Christensen's urea medium. Cerebrospinal fluid or serum cryptococcal antigen was detected using a CLA test (PASTOREXTM CRYPTO PLUS) following the manufacturer's instructions. The specimens were inactived by heating at 56 °C for 30mn. Prior enzymatic treatment of all samples was performed in order to eliminate interferences and enhance detection sensitivity. After preparation, agglutination card was placed on the shaker for 5 min (160 rpm), at room temperature (18–30 °C). A positive reaction was noted when we observed agglutination of the

latex particles with the test sample. Microscopic examination was made by adding one drop of India ink on the CSF specimen.

Data Analysis

The data were entered into Excel TM and analyzed with the TM R2.15.0 software (R Foundation for Statistical Computing, Vienna, Austria). Qualitative variables were described in terms of numbers, percentage of data provided and confidence intervals calculated at 95%. Statistical comparisons were made using the Chi square test or Fisher test depending on the conditions of applicability. The test was considered as significant if p is less than 0.05.

For evaluating the performance of Dynamiker Cryptococcal Antigen Lateral Flow Assay (LFA) in the detection of *Cryptococcus*, the sensitivity, specificity, positive and negative predictive values were calculated. They were expressed as percentages with confidence intervals at 95%. The microscopy after india ink staining and/or the cryptococcal latex agglutination test (CLAT) were considered as reference methods. The kappa coefficient to assess the level of correlation between the Dynamiker Cryptococcal Antigen Lateral Flow Assay (LFA) and the standards methods was calculated.

Results

A total of 39 study participants with available specimens were identified and were tested in routine diagnostic. The table 1 describes the demographic and clinical aspects of patients enrolled in this study. The age of the patients varied between 24 and 68 years with a mean at 44.5 years. There were more female (64.1%) than male (35.9%). The majority of the patients were HIV-infected with a mean of CD4 cell count at 73 cell count/mL. Biological sample (CSF, serum and urine) collected from patients were sent to the laboratory in majority (69.3%) for the screening of opportunistic infections including cryptococcosis.

India Ink microscopy was performed on 15 patient specimens (Urine and CSF), with 3 of 15 yielding *C. neoformans*. Culture on Sabouraud was also performed on 15 patient specimens (Urine and CSF) with 5 of 15 Fielding *C. neoformans.*

The Latex agglutination was performed on all the 39 patients with 9 positive results.

The new Dynamiker Lateral flow Assay was performed on the 39 patients with 12 positive results. The intensity of the band varied between patients presenting positive results. Out of the 12 positive specimens, the Dynamiker Lateral Flow Assay yielded 8 (66.7%) results with high intensity level, 1(8.3%) with medium level and 3(25%) with poor intensity level.

Conparaison of conventional tests have shown a good concordance between the India Ink and the culture (k=0.66) and an excellent concordance between the Latex agglutination and the culture (k=1) as described in Table 2. These results allowed us to choose the Latex agglutination as reference method to assess the performance of the Dynamiker Lateral Flow Assay in all the 39 patients.

Compared to the Latex agglutination, the Lateral Flow Assay yielded a sensitivity at 100%, a specificity at 75% and a Youden at 75. The percent of concordance was at 92.3% as described in Table 3.

These results show that the Dynamiker Lateral Flow Assay is a good test for screening cryptococcosis cases. However, three discrepancies results have been observed with 3 positives cases with the Lateral Flow assay but negative for the latex agglutination (Table 3).

The profile of patients with discrepancies results are presented in the Table 4. They are all female with CD4 count varying from 29 to 80. The intensity of the band obtained in the lateral flow assay were poor for these patients.

	N	%
Socio-demographic aspects		
Mean ± SD age, y	44.5 ±11.9	
Gender, no. (%)		
Male	14	35.9
Female	25	64.1
Immune Status		
HIV infected	36	92.3
Undetermined	3	7.7
CD4 cell count/mL, mean± SD	73 ± 47.1	
Clinical aspects		
Screening for opportunistic infections	27	69.3
Febrile meningeal syndrome	9	23.1
Respiratory symptoms	3	7.6
Specimens collected		
Serum	24	61.5
Urine	1	2.6
CSF	14	35.9

Table 1: Profile of patients enrolled in the study.

		Tests performed		Cohen's Kappa	95% Confidence Limits	Comment
		India Ink				
		Neg	Pos			
Culture	Neg	10	0	0.66	[0.258, 1.076]	Good agreement
	Pos	2	3			
		Latex Agglutination				
		Neg	Pos			
Culture	Neg	10	0	1	[1, 1]	Excellent agreement
	Pos	0	5			

Table 2: Correlation between conventional tests and culture.

		New Lateral Flow assay		Sensitivity (%); 95%IC	Specificity (%); 95%IC	Youden's J	Percent Agreement
		Neg	Pos				
Latex	Neg	27	3	100	75%	75	92.3
	Pos	0	9	[100, 100]	[50.5, 99.5]	[50.5, 99.5]	[83.9, 100.7]

Table 3: Performance characteristics of the Dynamiker Lateral Flow assay compared to the Latex Agglutination used as a reference standard.

Age	Sex	Immune status	CD4	Specimen collected	Latex	New LFA	Intensity of the band
57	F	VIH	50	serum	Negative	Positive	Poor
40	F	VIH	80	serum	Negative	Positive	Poor
40	F	VIH	29	serum	Negative	Positive	Poor

Table 4: Patient's profile with discrepancies between the new LFA and the latex.

Discussion

This study has shown the good performance of a new antigen lateral flow assay for the rapid diagnosis of cryptococcosis. During the study, the interpretation of all the strips was easy after 10-minute incubation, even though variation in the intensity of the specific band was observed. The test yielded an excellent sensitivity at 100% whether testing sera, urine or CSF. Similar results on the performance of the Dynamiker have been reported in the literature. Indeed, Chisale et al. And Kwizera et al. Have shown a sensitivity at 100% in the detection of cryptococcal antigen in body fluids in comparison with other LFA [15,16]. Regarding these results, the Dynamiker LFA could be used in the field as a good Point of Care diagnosis. The advantages of the LFA are multiple. There is no pretreatment, the cost of the test is low, the result is rapidly obtained and it requires low level of laboratory skills and expertise [17-20]. The LFA tool is also very useful in the diagnosis of cryptococcal meningitis in children. Kalla et al. Have reported a prevalence carriage of cryptococcal antigen at 6.12% in children using a LFA test [21]. Despite the good sensitivity level, the specificity is low. Indeed, the Dynamiker LFA was positive in 3 patients while the Latex agglutination test remained negative. For these three tests, with discrepancies results the intensity of the band was poor. This may correspond to subclinical forms whose diagnosis may be anticipated with the most sensitive test. It can also be due to cross reactions with other fungal pathogens. These discrepancies results have been described between the LFA and the LA [22]. The authors of this study have proposed some explanations including minor contamination, possibility of cross reactivity or error in the reading. So, the LFA has been considered as better than the LA. However, the LA has the advantage of yielding an antigen titre. Chen et al. In their study have shown that a serum cryptococcal antigen titre > 1: 1024 had a high positive predictive value of Cryptococcal meningitis [23]. This diagnosis tool could help to guide the treatment in case of difficulty to obtain the cerebrospinal fluid.

Regarding the clinical status, the majority of the patients tested in our study were HIV-infected. We did not test HIV

negative patients. In a study carried out in Turkey, authors have demonstrated that the Dynamiker was able to detect 11% of cryptococcal antigen in HIV patients but no case in HIV negative people [24]. According to them, the rarity of CM in immunocompromised patients and the low incidence of Crypococcosis in Turkey could be some explanations. In another study, the LFA and the LA have been found also to be less sensitive in HIV negative patients presenting a disseminate disease [13]. Regarding these results, additional studies are needed to assess more sensitive diagnosis tool in this group at risk.

Conclusion

In conclusion, the excellent sensitivity of the Dynamiker Lateral Flow Assay, its ease of use (practicality and reading), as well as the rapidity of obtaining results (less than 15 minutes) are fully appropriate for routine diagnosis of cryptococcosis in resource limited settings. However, due to the small number of tests assessed, it is important to perform additional tests particularly in non-HIV patients at risk of developing cryptococcosis.

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