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LATERAL FLOW ASSAY FOR CRYPTOCOCCAL ANTIGEN: AN IMPORTANT ADVANCE TO IMPROVE THE CONTINUUM OF HIV CARE AND REDUCE CRYPTOCOCCAL MENINGITIS-RELATED MORTALITY

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SUMMARY

AIDS-related cryptococcal meningitis continues to cause a substantial burden of death in low and middle income countries. The diagnostic use for detection of cryptococcal capsular polysaccharide antigen (CrAg) in serum and cerebrospinal fluid by latex agglutination test (CrAg-latex) or enzyme-linked immunoassay (EIA) has been available for over decades. Better diagnostics in asymptomatic and symptomatic phases of cryptococcosis are key components to reduce mortality. Recently, the cryptococcal antigen lateral flow assay (CrAg LFA) was included in the armamentarium for diagnosis. Unlike the other tests, the CrAg LFA is a dipstick immunochromatographic assay, in a format similar to the home pregnancy test, and requires little or no lab infrastructure. This test meets all of the World Health Organization ASSURED criteria (Affordable, Sensitive, Specific, User friendly, Rapid/robust, Equipment-free, and Delivered). CrAg LFA in serum, plasma, whole blood, or cerebrospinal fluid is useful for the diagnosis of disease caused by *Cryptococcus* species. The CrAg LFA has better analytical sensitivity for *C. gattii* than CrAg-latex or EIA. Prevention of cryptococcal disease is new application of CrAg LFA via screening of blood for subclinical infection in asymptomatic HIV-infected persons with CD4 counts < 100 cells/μL who are not receiving effective antiretroviral therapy. CrAg screening of leftover plasma specimens after CD4 testing can identify persons with asymptomatic infection who urgently require pre-emptive fluconazole, who will otherwise progress to symptomatic infection and/or die.

KEYWORDS: Cryptococcal meningitis; Cryptococcus; Diagnosis; Lateral flow assay.

INTRODUCTION

Cryptococcosis is a disease produced by species of *Cryptococcus*, which are unique in their capsular polysaccharides. This feature has been targeted for developing diagnostic tests that identify cryptococcal antigen (CrAg) (KOZEL & BAUMAN, 2012). Globally, most cases of cryptococcosis are caused by *Cryptococcus neoformans* (serotypes A and D). *C. neoformans var. grubii* (serotype A) has a worldwide distribution and accounts for more than 90% of all cases of cryptococcosis, particularly in persons with acquired immunodeficiency syndrome (AIDS) (LITVINTSEVA *et al*., 2011). *C. neoformans var. neoformans* (serotype D) also has a global distribution, but clinical cases are concentrated in Europe. *C. gattii* (serotypes B and C), has traditionally been recognized a "tropical or subtropical fungus". The spread of *C. gattii* infection into new geographic areas was heralded by an ongoing outbreak that began in Vancouver Island, Canada and then was observed in other regions of British Columbia and the northwestern United States. Historically, our understanding is that *C. gattii* causes disease predominantly in persons with apparently "normal" immune system. However, more recently, new risk groups have been recognized, including patients with human immunodeficiency virus (HIV) and other immunodeficiencies, particularly auto-antibodies against granulocyte-macrophage colony stimulating factor (GM-CSF) (CHEN *et al*., 2014).

Here, we will review the epidemiology of the current scenario of cryptococcal meningitis in HIV-infected persons, the strategies to reduce mortality and morbidity due to AIDS-related cryptococcal meningitis, and how CrAg lateral flow assay (CrAg LFA) can be useful in this context.

Global burden of cryptococcal meningitis in HIV-infected patients

AIDS-related cryptococcal meningitis causes approximately 15% of AIDS-related mortality annually (PARK *et al*., 2009). Sub-Saharan Africa has the highest burden, where *Cryptococcus* is the most common cause of adult meningitis (JARVIS *et al*., 2010; RAJASINGHAM *et al*., 2015), but Latin America is the third global region with most cases with 54,400 estimated cryptococcal meningitis cases annually in 2008 (PARK *et al*., 2009). More recent 2015 estimates of cryptococcal meningitis incidence in Latin America are approximately 10,000 cases annually (RAJASINGHAM, unpublished data).

There has been a massive expansion of access to antiretroviral therapy

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(ART) in Latin America. AIDS-associated cryptococcal meningitis has decreased dramatically in the ART era (GUIMARÃES 2000; MIRZA *et al*., 2003; DROMER *et al*., 2004; JARVIS & HARRISON, 2007; GRECO & SIMAO, 2007). However, the impact of ART in reducing cryptococcosis appears to be less in low and middle-income countries with suboptimal access to ART.

Mortality of AIDS-related cryptococcal meningitis

Currently, cryptococcal meningitis represents the main cause of HIV-related opportunistic meningitis in Brazil (OLIVEIRA *et al*., 2006; VIDAL *et al*., 2008; LEIMANN & KOIFMAN, 2009), and in most low and middle-income countries (HARRISON, 2009). Mortality continues to be unacceptable high. In retrospective and prospective hospital-based studies performed in Brazil and Argentina, the case fatality rates have ranged from 26% to 63% (METTA *et al*., 2002; PASQUALOTTO *et al*., 2004; MOREIRA *et al*., 2006; MÓNACO & TAMAYO ANTABAK, 2008; LINDENBERG *et al*., 2008; VIDAL *et al*., 2012). This figure is similar to 24 - 50% reported in interventional studies carried out in Africa and Asia (BICANIC *et al*., 2007; BICANIC *et al*., 2009; JARVIS *et al*., 2009; JARVIS *et al*., 2014). In contrast, mortality ranges from 2.5% to 25% in high resource countries (KAPLAN *et al*., 2000; DROMER *et al*., 2004; D'ARMINIO MONFORTE *et al*., 2004; DILLEY *et al*., 2005; LORTHOLARY *et al*., 2006; DROMER *et al*., 2007; ANTINORI *et al*., 2008). Thus, improved outcomes are possible.

Mortality in high-income versus low and middle-income countries

The differences in outcome between low and middle-income countries versus high-income countries have several potential explanations. First, late testers and late presenters with HIV infection are more frequent in low and middle-income countries (GRECO & SIMÃO, 2007). Low CD4 count at ART initiation and more advanced disease constitutes strong predictors of mortality in the first year of ART (TUBOI *et al*., 2009). Second, cryptococcal meningitis usually reveals HIV-infection in low- and middle-income countries but some studies showed other alternative scenario, particularly in middle-income countries: most cryptococcal meningitis are aware of their HIV-status prior to admission (with or without prior ART use) (VIDAL *et al*., 2012; MORA *et al*., 2012). This finding suggests continued missed opportunities to initiate or maintain ART with persistent barriers to adherence and retention-in-care of a subset of patients. With expanded ART access, virologic failure and AIDS-progression is beginning to become more frequent. Third, severe immunosuppression (also frequent in high-income countries) but particularly concomitant anemia, malnutrition, and severe cryptococcal meningitis are common in low and middle-income countries (VIDAL *et al*., 2012). The delay in presentation with diagnosis only when cryptococcal meningitis is advanced is common (VIDAL *et al*., 2012; WHO, 2011). This is evident by the increased proportions of patients presenting with neurologic complications in resource-limited setting (KAMBUGU *et al*., 2008; SCARBOROUGH *et al*., 2007). Fourth, rapid diagnosis is paramount to optimizing survival; however, diagnosis is difficult when optimum laboratory support is unavailable in most low and middle-income countries. CSF microscopy with India ink staining is most commonly used but misses 15-20% of the low burden infections which are easiest to treat (BOULWARE *et al*. 2014). Although larger urban centers have a reasonable laboratory infrastructure, the availability of a timely diagnosis is highly variable by country and within country. Fifth, optimal medical management is not available frequently in low and middle-income countries. Combination antifungal induction therapy and utilization of adequate measures to control of intracranial pressure are heterogeneous in routine practice.

Prognostic factors associated with mortality

Several risk factors for treatment failure or mortality have been reported elsewhere for AIDS-associated cryptococcal meningitis (DROMER *et al*., 2007; CHARLIER *et al*., 2008; BICANIC *et al*., 2009; JARVIS *et al*., 2014; VIDAL *et al*., 2012). The major risk factors for mortality include: fungal burden (i.e. assessable by quantitative microscopy, quantitative cultures, and/or cryptococcal antigen titers), rate of fungal clearance, altered mental status, paucity of CSF WBC pleocytosis, abnormal brain imaging, elevated intracranial pressure at admission (which is uncontrolled), lack of therapeutic lumbar punctures to control pressure, disseminated infection, and duration of antecedent symptoms.

Strategies to reduce mortality and morbidity due to AIDS-related cryptococcal meningitis

A number of strategies to reduce mortality and morbidity due to cryptococcal meningitis were reviewed elsewhere (VIDAL *et al*., 2013). Table 1 depicts some key potential strategies to reduce the effects of AIDS-related cryptococcal meningitis.

Table 1
Potential key points to reduce mortality and morbidity due to AIDS-related cryptococcal meningitis

1. Earlier HIV diagnosis and ART initiation with retention-in-care, prior to AIDS.
2. ART adherence and retention-in-care.
3. Pre-ART CrAg screening using lateral flow assay or latex agglutination.
   a. Pre-ART positive CrAg predicts future cryptococcal meningitis despite ART.
   b. Preemptive treatment with fluconazole 400 mg twice daily for 2 weeks, then 400 mg daily for 8 weeks, and then 200 mg/day until CD4 > 200 is recommended.
   c. Primary prophylaxis is less cost-effective than CrAg screen and treat approach.
4. Improved cryptococcal meningitis care using lateral flow assay or latex agglutination.
5. Improved cryptococcal meningitis care.
   a. Induction therapy with amphotericin B (1.0 mg/kg/day) plus flucytosine 100 mg/kg/day (in four doses). When flucytosine is not available, fluconazole 800-1200 mg/day (in two doses) is the alternative.
   b. Preemptive IV fluid and electrolyte (K^+, Mg^{++}) supplementation during amphotericin administration to decrease toxicity.
   c. Aggressive control of elevated intracranial pressure with therapeutic lumbar punctures.
   d. Quantitative microscopy can predict culture status and guide when to switch to consolidation therapy.

Lateral flow assay

Which the classical diagnosis of cryptococcal meningitis?

The diagnosis of cryptococcosis is relatively simple with several potential techniques: direct visualization, histopathology, culture, and detection of CrAg in bodily fluids (PERFECT & BICANIC, 2014). Direct visualization using India ink staining has been the traditional method for identifying *Cryptococcus* organisms, particularly in resource-limited settings. The sensitivity is ~80-85% in AIDS-related cryptococcal meningitis but can be highly variable and often operator dependent, as lysed leukocytes can be mistaken for fungal elements (MAKADZANGE & McHUGH, 2014). The Giemsa stain and Hematoxylin and Eosin are not very useful for the histopathological diagnosis of cryptococcosis. The histopathology of infected tissue with specific stains shows the cryptococcal capsule (mucicarmine and Alcian blue stains), presence of melanin (Fontana-Manson), or grocott's methenamine silver stain of the fungal cell wall (PERFECT & BICANIC, 2014). *Cryptococcus* can readily be cultured from most sites, using routine and automated systems. In AIDS-related cryptococcal meningitis, the sensitivity of CSF and blood cultures are ~90% and ~50-70%, respectively (ANTINORI, 2013). Interesting, culture sensitivity is dependent on CSF volume (~82% for 10 µL, 94% for 100 µL) (BOULWARE *et al.*, 2014). Detection of CrAg, a component of the organism's glucoronoxylomannan polysaccharide capsule, is the most sensitive diagnostic tool for cryptococcosis (MAKADZANGE & McHUGH, 2014; PERFECT & BICANIC, 2014). Detection of CrAg in serum and CSF by latex agglutination test (CrAg-latex) or enzyme immunoassays (EIA) has been available for over 35 years (PERFECT & BICANIC, 2014). Most comparative studies used cultures as gold standard. CrAg-latex serum sensitivity ranged from 83-97%. The tests with the lower sensitivity did not use pronase on serum specimens. The CrAg-latex specificity on serum ranged from 93 to 100%. The CSF sensitivity of the CrAg-latex test was high ranging from 93 to 100% with specificity ranging from 93-98%. The sensitivity and specificity of the EIA on serum were 94% and 96%, respectively. The sensitivity and specificity on EIA testing of CSF were 100% and 98%, respectively. Among persons with AIDS with meningitis, CrAg should be always evaluated, including when India ink fails to identify yeast. For example, in Uganda, the most common cause of meningitis among HIV-infected persons with a negative India ink is still *Cryptococcus* (BOULWARE *et al*., 2014). Although the CrAg-latex performs well when compared with EIA and culture, its main limitation is that latex is a cumbersome manual test with subjectivity in the interpretation of the result. CrAg-latex and EIA also requires laboratory equipment and refrigeration of reagents, making it unsuitable for use in settings with no or minimal infrastructure (MAKADZANGE & McHUGH, 2014). The need for refrigeration dramatically raises the cost of the test in resource-limited settings. CrAg-latex has reduced sensitivity for CrAg of serotype C (i.e. *C. gattii*), and EIA shows reduced sensitivity for CrAg of serotype C and D. False positives rate is less than 1% and generally is explained by technical issues or other infections or contamination (i.e *Trichosporon beigelii*, *Capnocytophaga canimorsus,* and *Stomatococcus mucilaginosus*). False negative results can occasionally be observed with early infections when there is low fungal burden, with prozone phenomenon, and with poorly encapsulated organisms (ANTINORI, 2013; PERFECT & BICANIC, 2014). Table 2 shows a comparison of CrAg LFA with other immunoassays.

Table 2
Comparison of lateral flow assay with other immunoassays*

Lateral flow assay: requires no pretreatment of sample; high sensitivity for CrAg of all serotypes; suitable for use in settings with no, minimal or advanced infrastructure; rapid results (10 minutes); low overall cost.

Latex agglutination: serum samples pretreated with pronase; reduced sensitivity for CrAg of serotype C; not suitable for use in settings with no or minimal infrastructure; rapid results (10-30 minutes, depending on specimen type); intermediate overall cost.

Enzyme immunoassay: requires no pretreatment of sample; reduced sensitivity for CrAg of serotypes C and D; not suitable for use in settings with no or minimal infrastructure; longer time to result (35-45 minutes); higher overall cost.

*Adapted from KOZEL & BAUMAN, 2012.

How does the cryptococcal antigen lateral flow assay works?

The CrAg LFA (Immuno-Mycologics, Norman, OK, USA) is a lateral flow immunochromatographic assay and was designed to meet two critical criteria for diagnosis: 1) the test is able to detect CrAg of all *Cryptococcus* serotypes, and 2) the test can be used in settings with minimal or no infrastructure (KOZEL & BAUMAN, 2012). The CrAg LFA addresses the needs of low-income countries and meets the World Health Organization (WHO) ASSURED criteria for diagnostic tests (Affordable: low cost; Sensitive: equal to or to better than other CrAg tests; Specific: similar to other CrAg tests; User friendly: in a format similar to the pregnancy test strip; Rapid/robust: produces a clear result in 10 minutes; Equipment-free: the test requires no electricity or laboratory infrastructure; and Delivered: the test is small, lightweight, requires no refrigeration and has a long shelf life).

The CrAg LFA uses monoclonal antibodies which allows for consistent reagent quality and performance. The LFA uses a combination of two monoclonals. One monoclonal antibody is highly reactive with CrAg of serotypes A, B, and C; the second monoclonal is highly reactive with CrAg of serotypes A and D. Used together, the antibodies are highly reactive with CrAg across the range of cryptococcal serotypes, an advantage when compared with CrAg-latex or EIA.

Figure 1 shows the mechanism of CrAg LFA. The LFA uses gold-conjugated, two monoclonal antibodies impregnated onto an immunochromatographic test strip. If cryptococcal antigen is present in a specimen, suspended, gold-conjugated antibodies bind to the antigen. The gold-antibody-CrAg complex migrates by capillary action up the test trip, interacts with immobilized monoclonal antibodies against CrAg, and forms a red line. The Immy CrAg LFA kit contains 50 CrAg LFA test strips, specimen diluent, titration diluent, and positive control. The kit can be stored at room temperature for ~2 years.

The CrAg LFA provides both qualitative and semi-quantitative, e.g. titer, results. The test requires five simple steps shown in the Figure 2. The presence of two lines (test and control lines), regardless of the intensity of the test line indicates a positive result. A single control line indicates a negative test result. If the control line does not appear, the

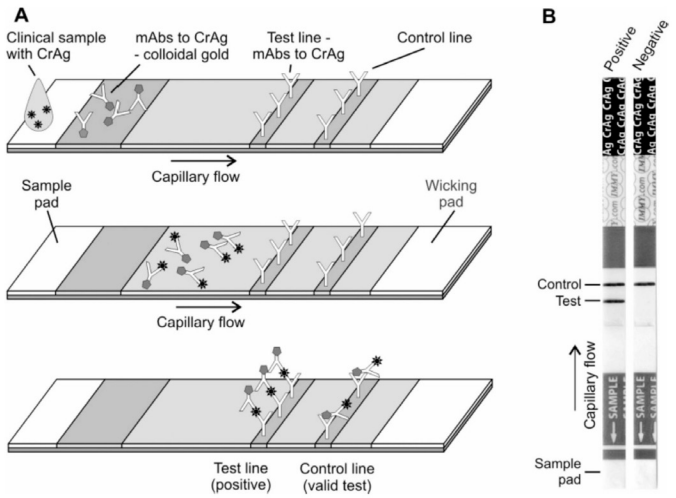

Fig. 1 - A. Schematic showing operation of lateral flow immunochromatographic assay for detection of cryptococcal antigen. **B.** Images of positive and negative lateral flow assay. (From KOZEL & BAUMAN, 2012).

results are invalid and the test should be repeated. For semi-quantitative results, the patient's titers are reported as the highest dilution that yields a positive test result. The CrAg LFA has been cleared by the U.S. Food and Drug Administration (FDA) for use with serum and CSF specimens. In addition, the test has received the *Conformité Européenne* (CE) Marking for serum, plasma and CSF. The CE Mark indicates that the assay conforms to the essential requirements of the European Conformity Directives. In Brazil, the test can be used with serum, plasma, and CSF.

Which the scenarios to use the lateral flow assay?

1. Screening and preemptive treatment for subclinical cryptococcosis

Several studies performed in Africa have reported the prevalence of detectable serum cryptococcal antigenemia between 2-21% in patients with CD4 < 100 cells/μL entering into HIV care (MEYA *et al*., 2010). Most studies about CrAg screening were performed with CrAg-latex testing. An isolated positive serum CrAg before ART predicts the development of cryptococcal meningitis, particularly when the CrAg titer is ≥ 1:8 (JARVIS *et al*., 2009; MEYA *et al.,* 2010; RAJASINGHAM *et al*., 2012). CrAg LFA is approximately 5-fold more sensitive than CrAg-latex when directly comparing semi-quantitative titers by serial dilution (BOULWARE *et al*., 2014). For example, a specimen positive at 1:8 CrAg titer by CrAg-latex is generally positive at 1:40 titer dilution by LFA. There is individual variation, however. In routine clinical conditions usually titration is not possible due to costs, thus, a qualitative result is enough to implement this strategy. Laboratories may consider running two dilutions at 1:160 and 1:1280 to perform titers to help risk stratify into low (< 1:160), medium (≥1:160), high burden (≥1:1280) of infection. The use of routine serum or plasma antigenemia screening in ART-naïve adults with CD4 < 100 cells/μL, followed by preemptive fluconazole therapy can reduce the development of cryptococcal meningitis and improve survival. The WHO recommended preemptive treatment for subclinical positive CrAg is fluconazole 400 mg twice daily for two weeks followed by 400 mg daily for eight weeks (WHO, 2011). This WHO recommendation works best for persons with low burden of infection (CrAg titer < 1:160). The necessity of secondary prophylaxis is unclear, but for higher burdens of infection (i.e. CrAg titers > 1:160), fluconazole 200 mg/day until CD4 > 200 cells/μL is advisable. This screening and treatment strategy is highly cost-effective, and is WHO recommended among HIV-infected adults not receiving effective ART where the prevalence of cryptococcal antigenemia is ≥ 3% (WHO, 2011). This is generally anyone with CD4 < 100 cells/μL worldwide; however what is the optimal threshold (i.e. 100, 125, 150, 200 cells/μL) for cost-benefit of screening is not known.

Currently, the Brazilian Cryptococcosis Network is performing a multicenter study using CrAg LFA in order to determine the CrAg prevalence among persons living with AIDS. In the meanwhile, seems reasonable to incorporate in clinical practice the screening and preemptive treatment for subclinical cryptococcosis strategy; however, CrAg screening implementation presents individual and public health challenges (MFINANGA *et al*., 2015; GOVENDER *et al.*, 2015). CrAg testing is recommended for pre-ART screening (WHO, 2011), and the CrAg LFA has several advantages over CrAg-latex or EIA for actual implementation of a screening program.

Some authors recommend that in centers where lumbar puncture is readily available, this procedure should be offered to all CrAg+ patients, but if it is not logistically feasible this should not be a barrier to the implementation screening (JARVIS *et al*., 2012). The role of lumbar puncture in asymptomatic CrAg+ patients is yet to be defined. Symptoms are not reliable for detection of dissemination to the central nervous system

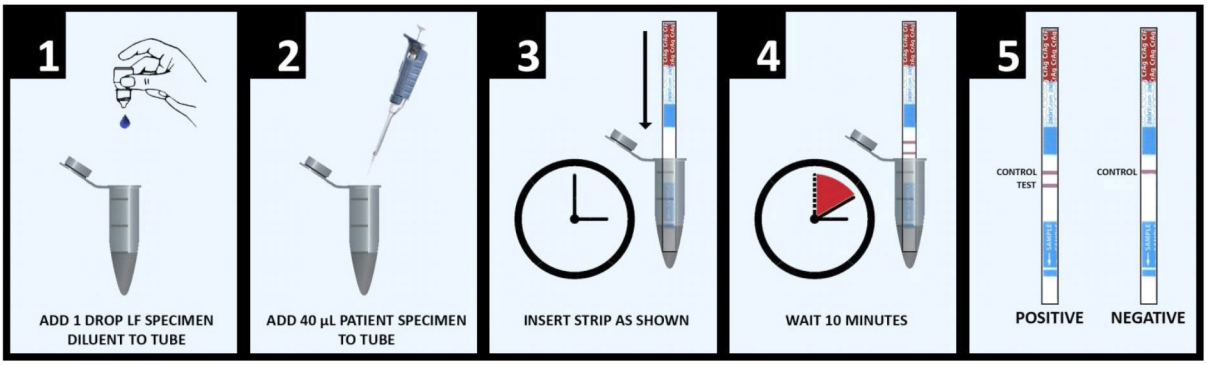

Fig. 2 - Five easy steps to perform the detection of cryptococcal antigen using lateral flow assay. Step 1: add one drop of specimen to a tube. Step 2: add of 40 μL (1 drop) of patient specimen to the tube. Step 3: insert the LFA strip into the tube. Step 4: incubate for 10 minutes. Step 5: interpret results. (From PELFREY & BAUMAN, 2012).

(WILLIAMS *et al*., 2015). Asymptomatic persons may have CSF positivity, and symptomatic persons may have CSF abnormalities but no diagnostic evidence of *Cryptococcus*. CrAg titer in blood can be quite helpful to risk stratify. In persons with low CrAg titers (< 1:160), they can be treated safely without lumbar punctures (unpublished data). Persons with high CrAg titers (≥ 1:1280) should be assumed to have disseminated disease, regardless of symptoms. The role of lumbar punctures to exclude active CNS cryptococcal disease has substantial variations in opinion. Excluding increased intracranial pressure in a symptomatic person is as important as to whether the CSF has detectable CrAg. We propose an algorithm for implementation of CrAg screening considering CrAg titres (Fig. 3).

2. Diagnosis of cryptococcal meningitis

Data from manufacturers indicate that LFA sensitivity, specificity, positive predictive value, and negative predictive value are 99.5%, 99%, 98%, and 99.7%, respectively, using serum, plasma, CSF or urine, when compared to culture (Table 3). Some studies validated LFA versus LA and EIA in both plasma and CSF samples in laboratory settings showing high values of concordance (JARVIS *et al*., 2011; LINDSLEY *et al*., 2011; BINNICKER *et al*., 2012; McMULLAN *et al*., 2012). A multi-site study prospectively validated LFA in patients with suspected meningitis in sub-Saharan Africa (BOULWARE *et al*., 2014). In this comparative study, LFA had the best performance (sensitivity 99.3%, specificity 99.1%) while CrAg-latex sensitivity (97-97.8%) and specificity (85.9-100%) varied between manufacturers. Interesting, CrAg LFA was the only CSF positive test in 1.5% of cases, which also had peripheral blood CrAg positivity.

CrAg LFA has been evaluated with paired specimens from patients with cryptococcal disease (Table 4). As expected, since the presence of CrAg is dependent on disease progression, CrAg was found in serum but not CSF of some patients, indicating non-meningeal cryptococcal disease. It was also found that CrAg concentrations in urine are lower than in serum, plasma, or CSF, as seen by urine LFA-negative results when serum, plasma, and CSF are positive. Urine LFA can have false positives, thus any positive result should always be confirmed in blood.

Diagnosis of cryptococcosis using urine or whole blood specimens is a true point-of-care test, accessible in any setting without laboratory. Urine or whole blood are still undergoing evaluation and are not yet FDA-approved. Available data about urine shows high sensitivity and specificity (JARVIS *et al*, 2011; PELFREY & BAUMAN, 2012). A recent study that reported 100% agreement between whole blood, serum and plasma LFA results demonstrated that fingerstick CrAg is a reliable bedside diagnostic test (WILLIAMS *et al*., 2015). Fingerstick LFA seems

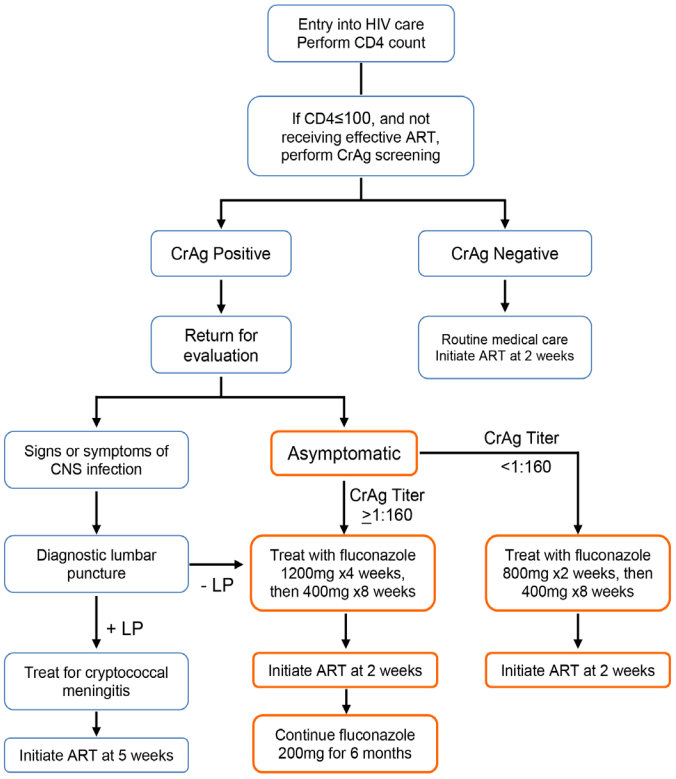

Fig. 3 - A proposal of an algorithm for clinical implementation of cryptococcal antigen screening and targeted preemptive therapy for the prevention of cryptococcal meningitis.

to be an important and simple tool for the diagnosis of cryptococcal meningitis. An instruction video on fingerstick LFA testing is available at: youtu.be/0RwN3p7XirQ

3. CrAg Lateral flow assay titres

High fungal burden and slow clearance of infection on treatment, together with altered mental status, are the most important drivers of acute cryptococcal-related mortality (JARVIS *et al.*, 2014). High fungal burden can be estimated with a baseline high titer of CrAg in serum or CSF (i.e CrAg-latex > 1:1024). In this line, baseline LFA titers correlate with quantitative cultures and predict 2- and 10-week mortality (KABANDA *et al*, 2014). Although CrAg titers generally decline gradually over the time at variable rates, older studies report that CSF, serum or plasma

Table 3
Sensitivity, specificity, positive predictive value, and negative predictive value of the lateral flow assay compared to cryptococcal culture*

Specimen Type	n	Sensitivity	95% CI	Specificity	95% CI	PPV	95% CI	NPV	95% CI
Serum	693	100%	98-100%	99%	97-99%	97%	94-99%	100%	99-100%
Plasma	135	100%	96-100%	100%	93-100%	100%	96-100%	100%	97-100%
CSF	261	100%	97-100%	97%	92-99%	97%	93-99%	100%	97-100%
Urine	674	98.4%	95-99.5%	99%	98-99.8%	97%	95-99.7%	99%	98-99.9%
Total	1763	99.5%	98-99.8%	99%	98.0-99%	97%	96-99%	99.7%	99.2-99.9%

*From PELFREY & BAUMAN, 2012.

Table 4
Lateral flow assay paired specimen comparisons from persons with cryptococcal disease*

Comparison	n	% Agreement Positive	95% CI	% Agreement Negative	95% CI
Serum vs. Urine	169	97.8%	94-99%	100%	89-100%
Serum vs. Plasma	83	100%	95-100%	100%	34-100%
Serum vs. CSF	118	93.7%	89-97%	100%	91-100%
Plasma vs. Urine	81	98.8%	93-100%	N/A	NB/A
CSF vs. Urine	104	98.5%	92-100%	91.7%	78-97%
CSF vs. Plasma	18	100%	82-100%	N/A	N/A

*From PELFREY & BAUMAN, 2012.

CrAg may remain positive for months to years, and CrAg titres are not recommended to be routinely measured to monitor response to treatment (POWDERLY *et al*., 1994). LFA presents similar limitation (KABANDA *et al*., 2014). CrAg tests and India ink are not useful for diagnosis of subsequent episodes of cryptococcal meningitis due to the possibility of long-term positivity (GOVENDER *et al*., 2013). Thus, a positive CSF culture for *C. neoformans* is the only one test to confirm mycological relapse or treatment failure (MUSUBIRE *et al*. 2013).

4. CrAg Lateral flow assay and Cryptococcus gattii disease

As mentioned above, the LFA uses two monoclonal antibodies impregnated onto an immunochromatographic test strip to detect CrAg for all four *Cryptococcus* serotypes which is an advantage in comparison with CrAg-latex or EIA (KOZEL & BAUMAM, 2012). Nevertheless, experience using the LFA to diagnose *C. gattii* disease is limited but it is likely to perform similarly as in *C. neoformans* disease. Further evaluation of its utility in the diagnosis of *C. gattii* diseases is needed, particularly in patients with extra-CNS or lung diseases (CHEN *et al*., 2014).

CONCLUSIONS

Despite relevant improvements during the ART-era, AIDS-related cryptococcal meningitis remains frequent and causes an unacceptable high mortality, particularly in low and middle-income countries. LFA allows a simple, rapid and low cost test for the diagnosis of cryptococcosis and is recommended for use with serum, plasma or CSF in symptomatic patients. In addition, CrAg LFA screening using plasma or serum has the potential to identify patients with asymptomatic infection who should receive pre-emptive fluconazole. Available data suggest that whole blood seems to be useful in these two scenarios. Thus, a fingerstick CrAg LFA represent a true point-of-care test to prevention and diagnosis of AIDS-related cryptococcosis.

RESUMO

Ensaio de Fluxo Lateral para antígeno criptocócico: um importante avanço para melhorar o *continuum* de cuidados com HIV e reduzir a mortalidade relacionada à meningite criptocóccica

A meningite criptocócica continua causando um substancial índice de óbitos em pacientes infectados por HIV em países de baixa e média renda. Ferramentas diagnósticas para detecção do antígeno capsular polissacarídico criptocócico (CrAg) em soro e líquor tais como o teste de aglutinação de látex (latex-CrAg) ou o imunoensaio (EIE) têm sido utilizadas por muitos anos. Técnicas diagnósticas mais aprimoradas seriam cruciais nas fases assintomática e sintomática da criptococose para reduzir a mortalidade. Recentemente, o ensaio de fluxo lateral para detecção do antígeno criptocócico (LFA CrAg) foi incluído no arsenal diagnóstico. Contrariamente aos outros testes, LFA CrAg é um ensaio imunocromatográfico em formato similar ao teste de gravidez, e requer pouca ou nenhuma infraestrutura laboratorial. Este teste preenche os critérios ASSURED (*Affordable*, *Sensitive*, *Specific*, *User friendly*, *Rapid/ robust*, *Equipment-free*, *Delivered*) da Organização Mundial da Saúde e pode ser utilizado em soro, plasma, sangue total ou líquor para o diagnóstico da criptococose. LFA CrAg tem melhor sensibilidade analítica para o *C. gattii* que o teste de látex-CrAg ou EIE. A prevenção da doença criptocócica constituiria uma nova aplicação do LFA CrAg, mediante a triagem de amostras de sangue para a identificação de infecção sub-clínica em pacientes infectados pelo HIV que não apresentam sintomas, possuem contagem de CD4 < 100 células/μL e não recebem terapia antirretroviral eficaz. A triagem de CrgA em amostras de plasma remanescente da contagem de CD4 pode identificar pacientes com infecção assintomática que precisam urgentemente de tratamento preemptivo com fluconazol, evitando assim a progressão para doença sintomática e/ou óbito.

CONFLICT OF INTEREST

Potential conflict of interest: the authors have not any link with the manufacturers and distributors of the laboratory tests mentioned in this paper.

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