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Diagnostic challenges of cryptococcus infection in HIV patients

Keywords: crytococcsis, cryptococcus infection, cryptococcal meningitis, cryptococcus antigen, immunological mycologics

Abbreviations: CNS, central nervous system; CM, cryptococcal meningitis; HIV, human immuno deficiency virus; IMMY, immunological mycologics

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Introduction

Crytococcsis is caused by *Cryptococcus neoformans* and *Cryptococcus gattii*.[1] This infection targets the central nervous system (CNS), in many cases causing Cryptococcal meningitis (CM). Annually, over 900,000 cases reported worldwide and two-thirds of those cases result in death.[1,2] Cryptococcosis has been reported as the most common opportunistic infection of the CNS.[3]This infection is particularly devastating in individuals with immune deficiencies[4] such as those suffering from HIV/AIDS; in fact, a growing number of CM patients (20-30%) are being diagnosed only after starting antiretroviral therapy for HIV/AIDS.[5] Early detection and treatment are key for survival from CM. This is possible as Cryptococcus antigen appears about 100days before the onset of symptoms.[2] However, with the currently available tests, early detection is not feasible or cost effective in resource-limited areas. The gold standard for CM detection has been the culture of patient's bodily fluids, but the poor sensitivity and the need for large sample volumes and laboratory facilities have made this method unreliable. Similarly, tests such as latex agglutination assays, are more effective than cultures, but are often not a feasible means for diagnosis in resource-limited settings because the procedure is costly, time intensive (can bedays before results are given to the patient), and requires facilities that can adequately handle, refrigerate, and process the samples before any actual tests can be carried out. More often, due to those constraints, patients remain undiagnosed either because of financial barrier or due to their inability to come back to clinic to receive their test results.[6] As patients from remote and rural villages cannot travel to clinics in urban due to time and cost involved in travel.

Recently, Immunological Mycologics (IMMY, Norman, OK, USA)[7] has developed a new method for the detection of the CrAg called the Lateral Flow Assay (LFA). Such an assay is inexpensive, can be processed quickly, and does not require technical expertise or any pre-treatment/storage of the samples before testing. In addition, the LFA may utilize serum, plasma, and CSF effectively to detect the presence of the Cryptococcal antigen, making it ideal test in resource-limited settings and in areas where HIV infection is fairly widely prevalent. The flexibility of this test and the speed with which it produces results could allow for the screening of Cryptococcal infection simultaneously with HIV monitoring as the excess of plasma from a CD4+ T cell count specimen can be used for the LFA. With a simpler diagnostic test such as this, screening could detect both symptomatic and asymptomatic CM (since CM antigen is higher in those individuals with an immune comprising infection such as HIV) allowing for not only the treatment of CM according to WHO guidelines but also the prevention of progressive symptoms through the use of fluconazole in asymptomatic patients (targeted prophylaxis). Such a methodology/diagnostic would make the test for the Cryptococcal antigen cost-effective both by reducing the cost of treatment as well as by limiting the number of hospitalizations needed.[5] In fact, a study conducted in Uganda to determine the cost effectiveness of such a diagnostic tool found that the cost to save one out of sixteen patients suffering from CM is 266 dollars, which is far cheaper than the average of 400 dollars required for the treatment for CM and also procedures for lumbar punctures, antibiotics, and checks for renal failure for about 2-week period.[2]

Infection with *Cryptococcus neoformans* has led to increased mortality and morbidity in HIV infected individuals. This has been widely seen in individuals with low CD4+ T Cell counts[2,8,9] from different parts of the world. Early identification of CrAg is highly necessary to prevent cryptococcal meningitis and its related expenses. This is important in a setting where overall > 4% of CrAg prevalence was seen in HIV infected individuals. Identification of these individuals is highly crucial in disease management. Because the symptoms are appear only at the final stages of disease. In HIV infected individuals Cryptococcus positivity was high in patients with <100 CD4+ T cell counts as witnessed in Thailand (13.1%),[10] Uganda (8.2%)[2] and South Africa (39%).[11] The recognition of CM symptoms is crucial in these individuals as it will increase the chances of carrying out CrAg screening early enough to detect the responsible fungus. But the status of asymptomatic individuals remains life threatening if left without CrAg screening and treatment. Therefore the researchers are recommending[9,12] CrAg screening for individuals with <100 CD4+T Cell counts, irrespective of their symptoms. Also the WHO Rapid Advice development group recommended CrAg screening mandated for the HIV positive antiretroviral (ART) naïve adults in population with high prevalence (≥3%) of Cryptococcal antigenemia.[13]

Apart from this, the kit sensitivity plays an important role in identifying the cryptococcus infected individuals, as direct microscopy and culture have 100% specific but poor sensitivity, the traditional latex agglutination assays also lacks some degree of sensitivity[14] there are new immunochromatographic test are[5,6,11] available which

gives good sensitivity and specificity but the usage of this kit must be expanded to different countries of the world where there is an absolute need for Cryptococcal detection like South Africa,[15] Nigeria,[16] India and Zimbabwe. Also other assays like the IMMY CrAg Lateral Flow Assay (LFA) (Immuno-Mycologics, Inc, Norman, USA) was found[17,18] to be more sensitive in detecting all subtypes of Cryptococcus antigens, and its broad reactivity makes it suitable for detecting the spectrum of Cryptococcal isolates. It uses the two types of monoclonal antibodies (mAbs)- one mAb is highly reactive with CrAg of serotype A, B and C; the second mAb is highly reactive with CrAg of serotypes A and D.[17] These monoclonal antibodies are directed against Cryptococcal polysaccharide capsule glucoronozy lomannan (GXM),[11] making the assay more sensitive. Apart from the sensitivity aspect, this assay is user friendly in following ways: there is no specimen pretreatment needed, the numbers of technical steps required are less when compared to latex agglutination assay. Furthermore, the test can be completed within one hour from the time of collection and it does not require any laboratory equipment; in addition, a very minimal specimen volume (10µL) is required to perform the assay. Apart from this advantages the Cryptococcal antigen detection kits must satisfy the WHO ASSURED (Affordable, Sensitive, Specific, User friendly, Rapid, Equipment-free, Delivered to those in need) criteria[6,17] and its recommendation to be used for the diagnosis of sexually transmitted infections and related pathogens.[19]

Another diagnostic challenge in Cryptococcal detection lies in the fact in the skills associated with the technical personnel performing the assay. As far as microscopy is concerned he/she should be able to correlate the identify the antigen, should go thoroughly all the fields in slide, in the culture techniques proper methodology must be followed to obtain fungal culture, in the latex agglutination assay proper specimen pre treatment procedures are to be applied to avoid false positive results. To overcome these challenges a user friendly assay like immunochromatographic technique must be available for the sensitive and specific detection of Cryptococcus infection. The widespread use of user friendly assay will improve the service provided to people in remote settings, who will not be able to travel to established clinical care settings for their medical care. Since the diagnostic tools such as India Ink/LA/EIA are unavailable outside the urban community. The advantages in rapid immunochoromatic tests like room temperature storage are highly valuable in resource limited settings like India where HIV infected individuals cannot travel to established hospitals for several reasons such as cost for travel, loss of wages, stigma etc.

Another challenge in diagnosis of Cryptococcal infection is the type of specimen required for collection, because neural involvement of Cryptococcal infection may not be detectable in serum or plasma specimens. Collection of CSF requires a physicians involvement, patient cooperation and hospitalization if needed. Diagnosis of Cryptococcus with immunochromatographic test early in asymptomatic and suspected individuals is cost effective in two ways. (i) This cost will be comparatively cheaper when compared to Latex Agglutination (LA) and ELISA assays. (ii) Early diagnosis of CrAg in asymptomatic individuals can help in better management of the condition and prevent neurological involvement thereby reducing the expenses which can incur due to future complications, administration of amphotericin, cost for hospitalization etc. Apart from this, the patients can get the report on the same day there by reducing the expenses to return to clinic (for obtaining results). Some patients may not come back to clinic to collect their results and this may lead to silent worsening of health conditions due to lack of treatment. A recent Ugandan study[2] confirmed that cost ($245) of amphotericin deoxycholate for the treatment of HIV associated CM without CrAg screening is more than the cost for CrAg screening and treating with preemptive fluconazole therapy in this population. This article emphasizes that the integration of CrAg screening must be mandated for the individuals initiating antiretroviral treatment especially with <100 CD4+ T Cell counts. Additional documented evidences are published by Jarvis et al.,[20] that demonstrated that CrAG screening among patient initiating ART in South Africa would be more cost effective to prevent CM related morbidity and mortality; the authors also recommend this strategy for other settings with high HIV and Cryptococcal meningitis (CM) incidence. A Cambodian study[21] compared the cost for CrAg screening for the individuals with <100 CD4+ T Cell counts against fluconazole prophylaxis in all these individuals. They found that targeted screening and treatment of clinical Cryptococcus is more cost effective than primary fluconazole prophylaxis for all the individuals. Hence it is essential that screening for CrAg be conducted for all antiretroviral naïve (especially with CD4+ T Cell <100 cells/cumm) HIV infected individuals and for the hospitalized patients. These literatures also highlight the importance of CrAg screening to prevent death among HIV infected individuals.[9,21–24] The rationale would be that with the high sensitive kits,[5,6,17] there would be a reduced instance to miss detecting this infection in the in-patient facility and with timely treatment, many lives can be saved. Hence CrAg screening must be mandated for the HIV infected individuals with CD4+ T Cell counts <100 cells/cumm. A reliable and cost effective kit must be implemented in all hard to reach populations around the world which is essential for the timely screening of this fatal opportunistic pathogen in a resource limited settings.

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Conflict of interest

The author declares no conflict of interest.

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