African Journal of Biotechnology Vol. 11(55), pp. 11760-11766, 10 July, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3854
ISSN 1684–5315 © 2012 Academic Journals

Review

An overview of diagnostic criteria for identification of cryptococcal meningitis with special emphasis on AIDS

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Accepted 26 June, 2012

Fungi are common in human immunodeficiency virus (HIV)-positive patients but HIV predisposes patients to several viral and bacterial infections that can result in meningitis. Understanding the burden of cryptococcal disease is particularly important for public health officials to adequately plan and prioritize needed resources for disease prevention and control. Cryptococcal meningitis, a fungal infection caused by *Cryptococcus* spp. is the second most common cause of opportunistic fungal infection in patients with acquired immunodeficiency syndrome (AIDS). It is important to define the burden of cryptococcal meningitis, as it relates to other important diseases, and to understand the need for public health attention to this infection. Frequently, HIV infection weakens the body's ability to fight disease. Infections which are rarely seen in those with normal immune systems are life-threatening to those with HIV. It is time to expand this global focus on HIV to include one of its most serious consequences, cryptococcosis. Few, if any, complications of advanced HIV disease have a greater influence on morbidity and mortality. We are likely to see little real progress in the outcome for these patients until there is a global commitment to invest in more drug availability, better access to easily used diagnostics and therapeutic devices, and more innovative clinical researches.

Key words: Cryptococcal meningitis, immune system, human immunodeficiency virus (HIV), serotrypes.

INTRODUCTION

Cryptococcal meningitis, a fungal infection caused by *Cryptococcus* spp. is the second most common cause of opportunistic fungal infection in patients with acquired immunodeficiency syndrome (AIDS) (Enoch et al., 2006). *Cryptococcus neoformans* is an important fungal pathogen causing invasive infection, especially of the central nervous system in this era of the human immunodeficiency virus (HIV)/AIDS epidemic (Scully et al., 2008). *C. neoformans* is encapsulated, ubiquitous environmental yeast that causes cryptococcosis, a potentially serious disease that affects healthy and immunocompromised individuals, especially patients with AIDS. Cryptococcal meningitis is a common opportunistic infection and AIDS-defining illness in patients with late -stage HIV infection, particularly in Southeast Asia and Southern and East Africa (Holmes et al., 2003; Chariyalertsak et al., 2001). Cryptococcal meningitis also occurs in patients with other forms of immunosupression and in apparently immunocompetent individuals. In parts of sub-Saharan Africa with the highest HIV prevalence, cryptococcal meningitis is now the leading cause of community-acquired meningitis, ahead of *Streptococcus pneumoniae* and *Neisseria meningitides* (Gordon et al., 2000). Mortality from HIV-associated cryptococcal meningitis remains high (10 to 30%), even in developed countries, because of the inadequacy of current antifungal drugs and combinations, and the complication of raised intracranial pressure (Van der Horst et al., 1997; Robinson et al., 1999).

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Abbreviations: HIV, Human immunodeficiency virus; **AIDS,** acquired immunodeficiency syndrome.

MICROBIOLOGY

There are two varieties of *C. neoformans* with different virulence: which causes human infection commonly *C. neoformans* and which cause disease in patients with

immune suppression and *C. gatti* that cause disease in normal hosts (Casadevall and Perfect, 1998; Gupta and Fries, 2010). There are five serotypes A, B, C, D and AD. *C. neoformans* comprises A, D and AD where as *C. gatti* comprises serotypes B and C (Heitman et al., 2006; Severo et al., 2009). These species and varieties may have different ecological characteristics, as well as a different epidemiology and pathogenicity. Virulence is due to production of oxidase and protease enzymes and the antiphagocytic properties of the carbohydrate capsule. Noncapsular mutant forms lack pathogenicity (Hogan et al., 1996). The significant differences in the ecology and epidemiology of the two varieties are discussed below. The clinical presentation of cryptococcosis due to the two varieties is generally indistinguishable, although one study suggests some possible distinctive features that are described below (Saul et al., 2008).

NATURAL HISTORY

A normally functioning host immune response is capable of eliminating this infection, or can confiscate *C. neoformans* into sites where it can remain controlled via fungi static and fungicidal host defence mechanisms.The humoral system is activated through the complement cascade. Therefore, the clinical manifestations of this infection can range from an asymptomatic colonization of the respiratory tract to a widespread dissemination depending on the host immune factors. As dissemination occurs, the central nervous system (CNS) is commonly involved. The basal meninges of the brain are preferentially affected causing thickening with subsequent invasion of the deeper brain tissues (Subramanian and Mathai, 2005). In the meninges, the organism appears to be suspended in a mucoid material that is derived from the capsule. Dissemination is due to serious defects in cell-mediated immune surveillance. Risk factors include: advanced HIV stage, corticosteroid use, lymphomas, solid organ transplant recipients, and patients with immune suppressive disease or receiving such drugs. Familial cryptococcosis has been reported and is due to genetic defects in the immune system (Krick, 1981).

EPIDEMIOLOGY

Fungi are common in HIV-positive patients but HIV predisposes to several viral and bacterial infections that can result in meningitis. Understanding the burden of cryptococcal disease is particularly important for public health officials to adequately plan and prioritize needed resources for disease prevention and control. It is important to define the burden of cryptococcal meningitis, as it relates to other important diseases, and to understand the need for public health attention to this infection. Frequently, HIV infection weakens the body's ability to fight disease (Adamson et al., 2009). Infections which are rarely seen in those with normal immune systems are life-threatening to those with HIV. Many opportunistic infections cause different severe affections such as several pulmonary diseases, chronic diarrhea, neurological defects, and different bacterial, parasitic and fungal infections. Meningitis associated with HIV/AIDS has an important impact on morbidity and mortality.

Before the AIDS epidemic, the incidence of cryptococcosis in the USA was less than one case per million persons per year. In the 1980s, cryptococcosis emerged as an important opportunistic infection amongst persons with AIDS, occurring in 5 to 10% of AIDS patients in the USA, Europe and Australia. With increasing use of fluconazole for oral candidiasis and the advent of highly active antiretroviral therapy (HAART) in the mid-1990s, the annual incidence of cryptococcosis decreased markedly in developed countries; in Atlanta, USA, it decreased from 66 cases per 1000 patients with AIDS in 1993 to seven cases per 1000 in 2000 (Mirza et al., 2003). In Southeast Asia and Africa, cryptococcosis appears to be relatively more common as an AIDS-related infection than it ever was in Europe or North America. In Thailand, cryptococcosis accounted for 19% of AIDS-defining illnesses between 1994 and 1998 (Chariyalertsak et al., 2001). In Uganda, the incidence of cryptococcal disease in patients with CD4 counts <200 cells/µl was estimated at 10.3 cases per 100 person years of follow-up (French et al., 2002). The high incidence of cryptococcosis in parts of Africa and Asia reflects differences in exposure rather than host susceptibility or cryptococcal strain virulence. As a consequence of the increase in HIV-associated cryptococcosis, there has been a shift in the epidemio-logy of meningitis; cryptococcal meningitis shown to be the leading cause of community-acquired meningitis, ahead of tuberculous and bacterial meningitis, accounting for 20 to 45% of laboratory-confirmed cases of meningitis in Southern Africa (Gordon et al., 2000).

Although, HIV-positive individuals are at increased risk of certain types of meningitis, typically cryptococcal and tubercular, evidence suggests that they are also more likely than the general population to develop community-acquired bacterial or viral meningitis. An early form of aseptic, HIV-associated meningitis develops within days to weeks after HIV infection. It appears as a mononucleosis-like illness and is rarely associated with encephalitis. Meningitides due to Cryptococcus, coccidioidomycosis, histoplasmosis, or other fungal infections are AIDS-defining events and occur typically with very low CD4+ lymphocyte counts. In Zimbabwe, Hakim et al. (2000) showed a decrease of purulent meningitis in adult hospitalised patients and an increase of tuberculosis and Cryptococcus meningitis. This was attributed to a rapid growth of this epidemic infection to HIV/AIDS. A good evolution of meningitis associated with

HIV/AIDS depends on different factors such as precocity of diagnosis, treatment, prescription and respect of specific drugs and immune status of the patient. Promising developments have been seen in recent years in global efforts to address the AIDS epidemic.

CLINICAL MANIFESTATION

Clinical diseases seen in HIV/AIDS patients are almost exclusively caused by *C. neoformans var. neoformans*, which is often serious and potentially life-threatening. Cryptococcal infections occur predominantly in patients with T-cell-mediated immune defects, especially those with AIDS and transplant-related immunosuppression (Soham and Levitz, 2005). Corticosteroid therapy and cancer chemotherapy are also predisposing factors for the development of cryptococcal infections (Perfect and Casadevall, 2006; d'Enfert and Hube, 2007). The incidence of cryptococcosis is also increased in subjects with hematologic malignancies, sarcoidosis, and diabetes mellitus. In immunocompromised patients, cryptococcosis can be severe and rapidly progressive, requiring prolonged systemic antifungal therapy. Cryptococcosis is uncommon in immunocompetent hosts, but it causes significant mortality and long-term morbidity. Mortality rates and morbidity vary by the etiology of meningitis and its values remain elevated. Higher mortality rates correlate with poor mental status, high cerebrospinal fluid (CSF) opening pressure at presentation, positive India ink test, extra-CNS manifestations, and higher fungal burdens. Studies have concluded that there are many virulent factors which attribute to the disease. Several factors have been identified that contribute to the virulence of C. *neoformans* strains. Studies of cryptococcosis in animal models infected with different strains of C. *neoformans* have indicated that there is considerable variation in the virulence of individual isolates (Boekhout et al., 2001). Immunocompetent patients with cryptococcosis tend to present with localised, indolent neurological disease and better clinical outcomes. On the contrary, cryptococcosis is a major fungal infection in HIV/AIDS patients. Its clinical presentation can be subtle, ranging from non-specific features of fever, malaise, sometimes, nausea and vomiting. Neck stiffness is an infrequent sign. Severe cases can present with encephalopathic features such as personality change and confusion, which also carry a worsened prognosis. Dissemination of the infection is common in AIDS patients, to e.g. liver and lymph nodes. Previous reports describing cryptococcosis in whom 10 to 40% were immunocompetent and suggest that host immune status may affect disease manifestation (Pappas et al., 2001). A higher proportion (30%) of meningitis patients have no predisposing factor identified, compared to patients who present with disease involving other sites (10 to 15%), and rates of extraneural involvement and fungaemia tend to be lower in immunocompetent patients presenting with meningitis (Hoang et al., 2004). Besides the immune status of the patient, the size of the inoculum is considered to be an important factor in determining the pathogenesis of this disease. It is believed that C. *neoformans* enters the human body via the respiratory tract. Elimination of C. *neoformans* is through cell-mediated immunity, with the participation of neutrophils, macrophages and cytotoxic T lymphocytes. In the face of immunodeficiency, control of the infection fails. The fungus may then disseminate to the central nervous system or other organs. However, the exact mechanism of dissemination remains unclear. Though cryptococcal meningitis carries a sinister prognosis and occurs mostly in patients with low CD4 count, routine antifungal prophylaxis is not recommended. This is because of the lack of survival benefit with primary prophylaxis, potential for development of resistance, possibility of drug interactions and cost (U.S. Public Health Service (USPHS) and Infectious Diseases Society of America (IDSA), 1999).

VIRULENCE

C. neoformans causes infection in individuals with defective T cell function, such as AIDS, as well as without underlying disease. It has been suggested that humoral as well as cellular immunity might play an important role in the immune response to *C. neoformans* infection. Various studies have shown that protein members of the heat shock protein (HSP) family, including the 70-kDa HSP (HSP70) from several pathogenic microbes, are antigenic (Swoboda et al., 1995). Moreover, HSPs of invading microbes are often the immunodominant targets of the cellular immune response as well as the humoral response. Other studies suggested that anti- bodies to HSPs from microbes play an important role in protection against infection (Zugel and Kaufmann, 1999; Bonnefoy et al., 1994). For instance, a 75-kDa immunodominant HSP has been observed in infections caused by *Chlamydia trachomatis* (Brunham et al., 1987). The protein is membrane bound and, like other members of the HSP70 family, may assist in the export and translocation of proteins (Zugel and Kaufmann 1999). However, little is known about the role of HSPs of *C. neoformans* in protection against cryptococcosis. Although secretory phospholipase has been demonstrated to be a virulence factor for C. *neoformans*, no conclusive correlations were made between virulence and phospholipase activities in clinical isolates.

The presence of Hsp90 in the capsule materials is an interesting finding. It has been demonstrated that the capsule is associated with virulence in *C. neoformans* and has been reported to have roles in pathogenesis as well. Therefore, HSP90 may have a role in the pathogenesis and virulence in *C. neoformans* (Nooney et al., 2005). On the other hand, the production

of HSP90 was noticed to be variable among the cells grown in the same growth conditions. Therefore, the productions of HSP90 in the cell are likely to be effected by other factors, such as gene expression and post-translation regulation. In addition, the presence of Hsp90 in the capsule materials helps us to use this protein as target for new antifungal therapy, such as a human recombinant antibody against HSP90 and it supports the idea of using HSP90 for serodiagnosis of cryptococcsosis (Cowen and Lindquist, 2005).

HOST DEFENCE

Goldman et al. (2000) has described a rat model that may reflect latent infection in an inherently resistant host such as man. In this model, pulmonary infection is controlled without dissemination, but viable cryptococcal cells remain for at least 18 months in interstitial granulomata within macrophages and epithelioid cells. Abrogation of immune control by corticosteroid administration results in extracellular yeasts and disseminated extrapulmonary infection. Much has been learnt about the immune response to cryptococcal infection from study of animal models and from *in vitro* experimentation (Shmuel and Stuart, 2005). In common with a number of other chronic fungal and bacterial infections, protection is associated with an active granulomatous inflammatory response, and depends on intact cell-mediated immunity involving both CD4 and CD8 cells, and a Th1 pattern of cytokine release. Protective roles for tumour necrosis factor-α (TNF-α), interleukins 12 and 18 (IL-12, IL-18) and interferon-γ (IFN-γ) have been inferred from experiments with knockout mice and antibody neutralization. More recent studies have begun to define how C. *neoformans* stimulates an innate immune response through interaction with Toll-like receptors on host cells (Yauch et al., 2004), and the cryptococcal mannoproteins that are important in stimulating specific T-cell immunity (Levitz et al., 2001).

DIAGNOSTIC CRITERIA

The laboratory diagnosis of cryptococcosis is established by the isolation of organism in culture, histopathology, or detection of its polysaccharide capsular antigen in cerebrospinal fluid (Tunkel and Scheld, 2000; Ennis and Saag, 1993). The organism grows in blood and chocolate agar within three to five days. Cytological examination (Papanicolou's stain) of sterile body fluids like CSF and India ink preparation for the negative staining characteristics are useful for identification of the organism. The latter is useful when >10 colony-forming units (CFU)/ml of yeasts are present (Subramanian and Mathai, 2005). Besides India ink, alcian blue, and mucicarmine are the other two stains used to detect the polysaccharide capsule of yeasts in tissue. Analysis of CSF usually reveals a poor white blood cell (WBC) count, inflammatory response, with a normal or low-CSF glucose levels, and a positive cryptococcal antigen test. The occurrence of cryptococcal antigen in the CSF (detected by latex agglutination) is not through a passive diffusion from serum but via active yeast invasion of the subarachnoid space.

The cryptococcal antigen detection test is not useful in monitoring the course of therapy. However, patients with lower titers (<1:8 in either serum or CSF) have better cure rates. Antigen titers can, however, vary depending on test kits used. In several patients, serum and CSF antigen titers remain high despite negative culture and good clinical response. In a retrospective review, it was found that although the serum antigen levels tend to decrease over time with therapy; this did not correlate with clinical response, persistent disease, or relapse (Aberg et al., 2000). Persistently high or unchanging antigen titers and a positive India ink preparation during the course of treatment or after, may suggest therapeutic failure or a relapse depending on the patient's clinical status.

RADIOLOGY

Although there are no characteristic chest radiography findings, single or multiple pulmonary nodules in immunocompetent patients and alveolar and interstitial infiltrates in AIDS patients are the most common chest radiograph abnormalities. CT scans may be normal or reveal meningeal enhancement, single or multiple nodules (cryptococcomas), cerebral oedema, or hydrocephalus (Biacanic and Harrison, 2004). Magnetic resonance imaging (MRI) scans are more sensitive for detection of multiple enhancing nodules within the brain parenchyma, meninges, basal ganglia and midbrain. In AIDS patients, there is often cortical atrophy and there may be coexistent pathology.

LABORATORY DIAGNOSIS

The rarity of cases of *Cryptococcus gattii* in immuno-compromised patients remains unexplained (Sorrell, 2001), in contrast to immunocompromised patients, few reports have described the variety of C. *neoformans*, clinical presentation, CSF parameters and prognosis associated with cryptococcal infections among immuno-competent individuals, contrast to infection caused by C. *neoformans*, *C. gattii* invokes a greater inflammatory response but carries a better overall prognosis (Lui et al., 2006). There is no consensus in the literature regarding criteria that could be used to distin-guish among proven cryptococcal infection, probable cryptococcal infection and colonization only. Diagnosis is rarely a problem in HIV-associated cryptococcal infection, since the high organism load means that Indian ink preparations of CSF

are usually positive, and cryptococccal antigen testing of either CSF or serum has a high sensitivity and specificity (Biacanic and Harrison, 2004).

MICROSCOPY OF CEREBROSPINAL FLUID

The CSF white cell count is raised, with a predominance of lymphocytes, in non-HIV-associated infection. In HIV-associated cryptococcal meningitis the CSF white cell count is lower and may even be normal (Biacanic and Harrison 2004). CSF protein is usually elevated and CSF glucose may be low. Indian ink examination is positive in 70 to 90% of AIDS patients but in only ~50% of non-AIDS patients. Lumbar puncture for CSF examination is indicated to diagnose or exclude meningitis, when the clinical picture is suggestive, or in the presence of extraneural disease or antigenaemia. Before the procedure, computed tomography (CT) scan must be performed to rule out space-occupying lesion especially when there are focal neurological signs or encephalopathic changes suggestive of raised intracranial pressure. Opening pressure of CSF is to be recorded as this is of prognostic significance. Encapsulated yeast in the CSF may be detected with the Indian ink stain. Serum cryptococcal antigen (CRAG) and fungal culture results of CSF are useful for confirming the diagnosis subsequently. CSF examination is important for both diagnosis and for predicting prognosis of cryptococcal meningitis. Unlike HIV negative patients, cryptococcal meningitis in AIDS patients can have normal protein/sugar and no pleocytosis in up to half of the cases (Shaunak et al., 1989).

CULTURE

C. neoformans from CSF, blood or other sites produces white mucoid (depending on the capsule thickness) colonies, usually within 48 to 72 h, on most bacterial and fungal (Sabouraud dextrose agar) media. Although C. *neoformans* grows at 37°C, a temperature of 30 to 35°C is optimal. Standard blood culture systems will detect cryptococcaemia. Identification is based on biochemical tests, such as for urease production, or DNA-based methods (Satish et al., 2010). On specialized media, such as birdseed agar, which contain diphenolic compounds, cryptococcal laccase leads to formation of melanin and brown colonies. Concanavine–glycine thymol blue agar can be used to discriminate *C. gattii* from *C. neoformans* isolates. Serotyping is possible with commercial kits using monoclonal antibodies (Tihana and Thomas, 2004).

SEROLOGY

Serologic tests for the detection of Cryptococcus are specific and sensitive (90%). The most common method for the detection of cryptococcal antigen is latex agglutination. Positive serologic results in the serum are associated with systemic dissemination, which increases the risk of evolving to central nervous system involvement, even in immunocompetent patients (Perfect and Casadevall, 2002). There is more extensive clinical experience with the serology testing of cerebrospinal fluid and blood, although detection of the antigen in urine and bronchoalveolar lavage fluid can also be used (Barbosa et al., 2006). Serodiagnostic can be applied in immune-suppressed patients, symptomatic immuno- competent patients and patients presenting positive serology for Cryptococcus, even for those presenting multiple nodules or extensive infiltrate, treatment is recommended. Clinical observation is reserved for isolated pulmonary cryptococcosis in asymptomatic immunocompetent patients presenting negative serology. The objectives of the treatment would be to promote rapid clinical resolution and to prevent the development of potentially fatal complications. Antibodies to *C. neoformans* are not useful in diagnosis. On the other hand, detection of the cryptococcal polysaccharide antigen in body fluids by rapid and simple latex agglutination tests or enzyme immunoassay has a sensitivity >90%, and at a titre of >1:4 is very specific. In addition to serum and CSF, urine and bronchoalveolar lavage fluid may be used. In asymptomatic HIV-infected patients, serum antigenaemia identifies early cryptococcal disease, requiring CSF examination and treatment (Bicanic and Harrison, 2004). High initial CSF titres (≥1:1024) correlate with a high organism burden by quantitative culture and are a marker of poor prognosis. CSF antigen titres fall with successful treatment, but are of little value in management. The imaging findings of cryptococcos is affecting the brain in immunocompetent patients can be different from the more commonly described findings in immune-compromised patients. Serum CRAG is an accurate and sensitive predictor of cryptococcal meningitis in advanced patients with fever. It has been shown that 98% of patients with culture-proven cryptococcal meningitis have a positive serum CRAG. A positive titer >1:8 should be taken as a presumptive diagnosis of cryptococcal meningitis.

HISTOLOGY

Since the clinical presentation of isolated cryptococcosis is unspecific and the radiological profile is non-pathognomonic, it is important to clarify the differential diagnosis. Cryptococcosis can be diagnosed by the direct study of the fungus in sputum, bronchoalveolar lavage, spinal fluid and histologic samples. It is confirmed by cultures from these samples (Barbosa et al., 2006).

TREATMENT OF CRYPTOCOCCAL MENINGITIS

The treatment for cryptococcal meningitis has been

established in the literature, there are no randomized, controlled studies defining the best therapeutic approach for immunocompetent patients. The impact of immune reconstitution in AIDS-related cryptococcal meningitis is unclear. Experience of discontinuing maintenance in this situation has been minimal and no recommendation can be made. Suffice to recap that cryptococcal meningitis can definitely be fatal, either at around time of acute diagnosis or for chronic refractory cases. Extreme caution has to be exercised and adequate treatment given for all cases to ensure control of the infection. Chronic suppressive treatment for life is required after induction treatment of cryptococcal meningitis as relapse rates of 50 to 60% and shortened survival have been found for patients not receiving such maintenance. Definitive diagnosis is confirmed by the culture of specimens, often the CSF or blood, and sometimes in respiratory secretions (Aberg et al., 1999).The primary objectives of treating cryptococcal meningitis are relief of symptoms and signs, control of infection, decrease in early mortality, prevention of relapse and maintenance of patient's quality of life. The optimum therapy for cryptococcal meningitis in patients with the AIDS remains unresolved. Traditional therapy consists of amphotericin B with or without flucytosine (Zeind et al., 1996). Amphotericin B and fluconazole are the two drugs found to be most effective in treating AIDS-related cryptococcal meningitis (Saag et al., 1992). 5-Flucytosine adds to the marrow toxicity of amphotericin B and such combination may not be well tolerated, especially in advanced disease. High-dose amphotericin B, with or without 5-flucytosine, followed by fluconazole is the standard induction treatment for acute cryptococcal meningitis. If necessary, itraconazole can be employed as the alternative for consolidation and maintenance therapy but it is presumably less effective than fluconazole. For very mild disease with normal mental state, some authorities suggested that amphotericin B might be omitted and fluconazole given alone. Evidence for this approach is generally not adequate. Response to acute antifungal treatment should be monitored against clinical, biochemical and microbiological parameters. Toxicity of drugs should also be monitored. For example, fluconazole inhibits cytochrome P450 hepatic enzymes, increasing levels of drugs such as rifabutin, terfanadine and cisapride. As a result, the risk of uveitis from rifabutin and cardiac arrhythmia from terfanadine and cisapride will be increased with concomitant fluconazole.

CONCLUSION

In summary, due to the severity of this infection and the potential life threatening condition that it represents, clinicians must be aware that cutaneous lesions may be one of the first manifestations of cryptococcosis caused by *C. gattii* especially in immunocompetent patients living and coming from endemic areas.

It is time to expand this global focus on HIV to include one of its most serious consequences, cryptococcosis. Few, if any, complications of advanced HIV disease have a greater influence on morbidity and mortality. We are likely to see little real progress in the outcome for these patients until there is a global commitment to invest in more drug availability, better access to easily used diagnostics and therapeutic devices, and more innovative clinical research. HIV-associated cryptococcosis is the elephant in the parlor, and it has been ignored for much too long.

More work is needed to develop simple to use, point-of-care diagnostic tests for cryptococcal meningitis, to develop improved initial treatments and to define the optimal timing. There is also the exciting possibility that much cryptococcal meningitis -related morbidity and mortality could be prevented by routine cryptococcal antigen screening of asymptomatic patients.

ACKNOWLEDGMENT

The authors are thankful to Vice-Rectorate for graduate studies and research for their kind support and co-operation.

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