Clinical manifestations and management of cryptococcal infection

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ABSTRACT

Cryptococcus neoformans is an important fungal pathogen causing invasive infection, especially of the central nervous system in this era of the HIV/AIDS epidemic. The choice of treatment depends on site(s) of infection and the patient’s immune status. Use of appropriate antifungal agents decreases mortality significantly, but requires continued therapy and long-term maintenance to prevent relapses. The use of liposomal amphotericin B (L Amp B) has overcome some of the difficulties usually found in this setting. The major advantage of these liposomal formulations is faster clearance of C. neoformans [cerebrospinal fluid (CSF) negative] and a reduction in amphotericin toxicity. The majority of clinical efficacy data related to L Amp B are derived from compassionate use studies and case series. Use of liposomal amphotericin has also shown to be a cost effective approach.

KEY WORDS: AIDS, amphotericin B, Cryptococcus, fluconazole, meningitis

Cryptococcus neoformans is the second most common cause of opportunistic fungal infection in patients with AIDS. Although first isolated in the juice of a fruit by San Felice in 1894, it was shown subsequently in 1901 to be present in natural sources like milk. It has been known to be a pathogen in humans for over 50 years. Various types of soil contaminated with pigeon extracts provide the environmental source of this widely prevalent organism. The high incidence of Cryptococcal meningitis among patients with HIV in Thailand is thought to be explained by the increased shedding of the organism in chicken droppings.[1]

Mycology

There are two varieties of C. neoformans with different virulence: C. neoformans var neoformans consisting of serotypes A and D, which cause disease in patients with immune suppression and C. neoformans var gatti consisting of serotypes B and C, that cause disease in normal hosts. The organism is usually 4–6 mm in diameter, but in the encapsulated form it can be up to 30 mm.


Natural history

Infections occur through inhalation of small diameter (less than 10 mm) yeast like organisms which enter smaller respiratory passages and then remain dormant depending on the host reaction.[5] Organisms are then reactivated from such previous dormant infections in the lung or lymph node, just as in tuberculosis (TB).

A normally functioning host immune response is capable of eliminating this infection, or can sequester C. neoformans into sites where it can remain controlled via fungistatic and fungicidal host defense mechanisms. The humoral system is activated through the complement cascade.[6] 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. 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.[7]

Laboratory diagnosis

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 (CSF). The organism grows in blood and chocolate agar within 3–5 days.[8]

Cytological examination (Papanicolaou’s stain) of sterile body fluids like CSF and India ink preparation for the negative stain-
ing characteristics are useful for identification of the organism. The latter is useful when >10^7 colony-forming units (CFU)/ml of yeasts are present. Besides India ink, alcin blue, and mucicarmine are the other two stains used to detect the polysaccharide capsule of yeasts in tissue.

Analysis of cerebrospinal fluid (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.\textsuperscript{[10]}

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.\textsuperscript{[11]} 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.\textsuperscript{[12]} 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.

Imaging techniques like computed tomogram (CT) or magnetic resonance imaging (MRI) of the head are used for detecting complications such as, hydrocephalus or mass lesions, where surgery may be indicated.\textsuperscript{[13]}

**Clinical presentation**

The infection occurs more commonly in men, possibly due to their occupational exposure or a lack of estrogens.\textsuperscript{[14]} Children are less commonly affected (less than 100 reported cases). But this pattern is likely to shift with increasing prevalence of HIV-infected children.

The body sites affected range from an asymptomatic pulmonary involvement, a meningoencephalitic form, or a wide dissemination into multiple body sites.\textsuperscript{[15]} Predominantly affected is the CNS and therefore it is essential that severely immunosuppressed patient be screened for an occult meningogal site even if the CNS is not the primary site of infection clinically.\textsuperscript{[16]}

**Brain**

Involvement of the parenchyma of the brain and menings occurs in 40–86% of patients as the organism has this unique and unexplained predisposition to establish an infection at these sites.\textsuperscript{[17]} The presentation can be acute or chronic. Nonmeningeal or nonparenchymal brain involvement is uncommon. An acute onset (<7 days) or chronic (>30 days) onset with headache, fever and nuchal pain suggesting meningeval irritation, occurs as the presenting symptom. However, neither headache nor fever need always be present in HIV-infected patients. Instead they could present with lethargy, obtundation, stupor, coma, or even dementia.\textsuperscript{[18]} Clinical signs lack precision or accuracy in predicting the aetiology in meningitis. Papilloedema may be observed but focal neurological signs such as cranial nerve palsies are rare. Intracerebral, cerebellar, and spinal cord lesions, although rare, have been noted.\textsuperscript{[19]} Complications of CNS involvement include intracellular hydrocephalus, focal motor deficits, changes in mentation, and symptoms of raised intracranial pressure. As lesions of brain parenchyma present as a ring-enhancing lesion on imaging, it can mimic *Toxoplasma gondii*.

Coinfection with other pathogens causing CNS infection, notably *Mycobacterium tuberculosis*, is reported as a common occurrence in patients with HIV. However, in a prospective study conducted among 105 patients diagnosed to have cryptococcal meningitis at our center, TB infection was not detectable in the CNS or at other sites.

**Lung**

*Cryptococcus* is the second most common community acquired fungus (excluding *Pneumocystis jiroveci*) isolated from the lung after *Aspergillus* sp. Although the lung is most likely to have been the initial portal of entry and infection, disseminated foci elsewhere with a normal chest X-ray can be seen. It is considered to be a reactivation of prior pulmonary infection (asymptomatic or subclinical carriage), analogous to TB reactivation.\textsuperscript{[20]} A primary pulmonary complex, similar to TB may be seen, or may be infected with TB.\textsuperscript{[21]} The localized pulmonary form is found as a solitary or as multiple small nodules in an asymptomatic individual on chest roentgenogram mimicking malignancy and requiring the diagnosis to be confirmed by lung biopsy. Symptoms of acute pneumonia with cough, fever, and lobar pulmonary infiltrates affecting alveoli or a diffuse interstitial pattern, indistinguishable from *P. jiroveci* infection occurs in patients with AIDS.\textsuperscript{[22]} Very rarely, it can present as an acute respiratory distress syndrome. Pleural effusion without parenchymal lesion is rare.\textsuperscript{[20]} Surgical intervention for a cryptococcal lung lesion is best avoided as local and systemic dissemination can occur.\textsuperscript{[21]}

**Other organ systems**

In the skin, cryptococcosis occurs as papules, abscesses, cellulitis, acniform lesions, draining sinuses, or subcutaneous swellings.\textsuperscript{[23]} Skin biopsy is required to confirm the diagnosis, and to differentiate it from molluscum contagiosum, Kaposi’s sarcoma, and herpes virus infection. Although, in a laboratory accident cryptococci can be injected directly into the soft tissue causing localized infection, the vast majority are due to dissemination from the lung.\textsuperscript{[23]}

Ocular involvement is seen as choroidal infection and endophthalmitis\textsuperscript{[24]} and can be confused in patients with AIDS with other coinfections such as: cytomegalovirus, *T. gondii*, or herpes simplex.

Occurrence of osteomyelitis and arthritis,\textsuperscript{[25]} and reports of direct traumatic inoculation into the bone\textsuperscript{[26]} have been reported. Cardiac valvular involvement, both of the native\textsuperscript{[27]} and pro-
thetis valves, mycotic aneurysms, and myocarditis can occur and sometimes it may cause sudden death. Enlarged adrenal glands on CT imaging of the abdomen should alert the clinician for a possible adrenal insufficiency (or associated histoplasmosis). Cryptococcal pyelonephritis and prostatitis have been reported. However, there have been no reports of sexual route of acquisition of infection.

Rarely, areas of the mucous membranes of mouth, larynx, anal region, skeletal muscle, and placenta have been reportedly infected. Although cryptococci can be isolated from the faeces, the gastrointestinal route of acquisition is unlikely and its affection besides hepatitis is not known. Hepatitis and peritoneal infection could present as an acute abdominal emergency.

Cryptococcosis and AIDS

It is the first opportunistic infection that occurs in over a quarter of patients who develop AIDS. About 5–10% of patients with AIDS (CD4 lymphocyte count of <200 cells/ml) develop cryptococcal meningoencephalitis, but this has decreased with the prophylactic use of triazoles antifungals such as fluconazole. About three quarters of patients have a large burden of organisms, evidenced by a highly positive CSF India ink preparation and very high titres of cryptococcal antigen in blood and CSF. The serum cryptococcal antigen test is generally positive in AIDS patients with CNS involvement and serves as a rapid screening test for patients suspected to have cryptococcal infection.

Treatment

Although treatment of cryptococcal meningitis is one of the best-studied infections, there is disparity in response rates noted. Choice of the antifungal agent depends on the site of infection, immune status of the patient and is among the polyene amphotericin B (Amp B), azoles (fluconazole, itraconazole), and flucytosine (FC).

Drugs of the azole class used previously were miconazole and ketoconazole. As these penetrate poorly into the CSF, achieve low levels in other tissues and have significant drug–drug interactions, it is not an ideal choice. Flucytosine, if used as monotherapy, results in the organism developing resistance and hence always used in combination with the polyene Amp B, where it has a synergistic effect.

Treatment of cryptococcal meningitis with Amp B monotherapy (0.4 mg/kg/day) given intravenously (IV) for 6 weeks (2–2.5 g total dose) clears the fungus in over half the patients treated, but dose-related nephrotoxicity may occur. In an effort to decrease Amp B toxicity, liposomal Amp B (L Amp B) was developed. Kshirsagar et al. have developed an indigenous patient worthy sterile pyrogen-free liposomal preparation (Fungisome™), which have shown to be safe and effective in cryptococcal meningitis in the dose of 1–3 mg/kg/day. As compared to conventional Amp B, this preparation is infused over a much shorter period requiring a smaller volume, does not require premedication and found to be safe in patients who had developed serious unacceptable toxicity with conventional Amp B. This patient worthy formulation is safe, efficacious, and cheaper than the commercially available formulation of Amp B. Combining Amp B (0.5–1 mg/kg/day) and oral FC (150 mg/kg/day) for 2 weeks as induction therapy followed by oral fluconazole 400 mg/day for 10 weeks is considered the standard treatment regimen for cryptococcal meningitis. This dual therapy has shown sustained clearance of cryptococci from CSF (60%) as compared to Amp B alone (51%). Addition of FC causes side effects like diarrhea, hepatitis, and bone marrow suppression usually in the first 2 weeks in a third of patients. Either, per orally administered itraconazole (400 mg/day) or fluconazole (400 mg/day) for 8 weeks, after initial Amp B therapy, had similar efficacy (70 and 68%). In such patients, dose reduction to 100 mg/kg/day could minimize these. Dose needs to be adjusted for renal dysfunction (creatinine > 2 mg%) and with serum FC levels measured by bioassay, enzymatic means, or by high-pressure lipid chromatography. Longer duration of FC therapy correlates with greater recurrence-free survival.

Fluconazole at 400 mg per day given orally or IV has excellent CSF penetration (60–80% of serum levels achieved) that is found even in the absence of meningeal inflammation. Monotherapy with oral fluconazole at doses of 400 mg daily achieved equivalent success rates with clinical cure in 63% and mycological clearance in cultures within 60–90 days in 76% in African patients with AIDS. Higher doses of up to 800 mg daily may be used in those who fail to improve.

Cryptococcus resistant to Amp B and/or fluconazole are rare, but emergence of resistance to fluconazole has become a concern, and is probably linked to extended maintenance regimen. Over two years (2000–2002), in a laboratory-based surveillance reported from Cambodia, the fluconazole MIC₉₀ rose 8-fold to 96 mg/l with resistance rates increasing from 2.5 to 14%. However, the MIC₉₀ for Amp B remained stable over the period.

Itraconazole penetrates the CSF poorly (like Amp B), but its hydrophobicity ensures drug accumulation in the host cells. Itraconazole combined with oral FC (150 mg/kg/day) had achieved responses in 12 of 13 patients, and appeared to shorten time to cure in a small study. However, in AIDS patients with cryptococcal meningitis, itraconazole alone is less effective than Amp B and FC. Itraconazole at doses of 200–400 mg/day for 4–6 months is used to treat individuals who cannot tolerate fluconazole.

Concern about the azole-polyene antagonism and inadequate clinical data in humans has precluded combination of Amp B with fluconazole. In a recent small trial conducted in Thailand, the combination of Amp B with FC cleared cryptococci from CSF of HIV-infected patients significantly faster as compared to Amp B alone, or in combination with fluconazole, or use of all three drugs simultaneously.

Intraventricular Amp B therapy has been used in patients with...
a poor prognosis, but the complications associated with multiple injections into the lumbar or cisternal space via subcutaneous reservoirs discourage this intervention. Cryptococcomas of the CNS that present as brain abscesses or mass lesions may rarely require operative intervention.\[15\]

Although chronic corticosteroid and cyclosporine therapy can predispose patients to cryptococcal infection and impair treatment response, the dose of these drugs need to be modified based on the underlying disease conditions for which they are being used. Patients with previously treated cryptococcal infection can receive solid organ transplantation without reactivating the fungal infection and need for antifungal prophylaxis.\[56\] Although cryptococcal meningitis has been reported only rarely in pregnant women, both Amp B and FC cross the placental barrier and no case of congenital cryptococcosis has been described.\[67\]

With Amp B usage, whole blood counts and serum electrolytes need to be monitored weekly. Demonstration of CSF clearance by culture negativity at the end of the second week of therapy is required. Re-examination of the CSF is mandatory in patients who deteriorate and should be repeated at the end of therapy, and at 1, 3, and 6 months post-therapy to detect relapses. Serially done lumbar punctures with drainage of fluid may also serve to reduce the headache in patients with raised intracranial tension (ICT). In a large study, patients with a pretreatment opening pressure <250 mm CSF had a better short-term survival than those with higher pressures (>250 mm). Furthermore, lumbar punctures to bring down the pressure by 10 mm in patients with opening pressures of >250 mm was shown to improve clinical response at 2 weeks.\[65\] However, it should also be kept in mind that at very high-opening pressures, there is a possible risk of cerebellar herniation occurring.\[69\] Drugs have not been shown to be effective in decreasing the intra cranial pressure. In a small study, the diuretic acetazolamide use was found to have higher risk of adverse effects.\[59\] There is no data on the utility of steroids in this condition.

Computed tomogram scan to identify hydrocephalus or presence of parenchymal lesions, as the cause of raised ICT may be required. Surgical options are reserved for patients with hydrocephalus or for those with intracerebral lesions. However, in a retrospective review, placement of intracerebral shunt in HIV negative patients with cryptococcal meningitis was found not to be useful.\[35\]

Lipid-associated amphotericin B formulations are being studied in the treatment of cryptococcal meningitis. Successful treatment outcome in patients with AIDS has been reported. In a retrospective review of the collaborative exchange of antifungal research (CLEAR), use of Amp B lipid complex (ABLC) in a median dose of 4.3 mg/kg/day, achieved a cure in 41 of 47 HIV-infected patients with CNS cryptococcal infection when administered for a median of 13 days.\[32\] In a randomized open-label trial from Burundi, the use of intralipid Amp B was associated with significantly lower chance of infusion related toxicity (fever and chills) but higher risk of nephrotoxicity as compared to Amp B in dextrose. There was no difference in cure rates between the two groups in this study.\[39\] In an open label, randomized, comparative phase III safety, and efficacy study with conventional Amp B and L Amp B in patients with systemic fungal infections, use of Fungisone in dose of 1–3 mg/kg/day achieved significant cure in cryptococcal meningitis.\[14\]. In phase II studies, L Amp LRC-1 (Fungisome) achieved a cure in six (85%) of seven cryptococcal meningitis patients.\[39\] There have been case reports from India of successful treatment of cryptococcal meningitis and long-term recurrence-free survival with liposomal formulations of Amp B (L Amp LRC-1).\[15\] Newer agents like caspofungin and voriconazole have not been tried in clinical trials for this infection. The role of cytokines to enhance host defense and immunomodulation with transfer factor are under study. A defective production of proinflammatory cytokines - interferon (IFN)-γ and tumor necrosis factor have been seen in patients with cryptococcal meningitis. In a recent study, patients with cryptococcal meningitis randomized to receive IFN-γ or placebo along with standard therapy (Amp B alone or with FC) showed that patients receiving IFN-γ had a significant 2-week culture conversion with a trend toward better mycological and clinical success. Further studies are required to support this, especially as the therapy is well tolerated.\[16\]

Treatment of asymptomatic pulmonary colonisation is unnecessary and at the other end of the spectrum, immunocompromised hosts with cryptococcal meningitis require definite and aggressive therapy. In case of symptomatic pulmonary infection, urinary tract, or cutaneous disease, fluconazole (200–400 mg/day) for 36 months is recommended.

### Prevention of relapse

Rapid institution of antifungal treatment reduces mortality. The duration of initial therapy is an area of controversy. Clinical trials suggest that patients with AIDS should get drugs for a longer duration. Relapse rates range from 15 to 25% in non-AIDS patients, and >50% in AIDS patients.\[66\] Chronic suppressive therapy is now the standard of care in such patients, and oral fluconazole is the most widely used drug. Intravenous Amp B (50 mg) once or twice weekly is effective. Comparative trials to evaluate the long-term management of cryptococcosis among AIDS patients are needed to improve outcome.

Fluconazole at 200 mg daily is recommended for chronic suppression as secondary prophylaxis for patients with AIDS. In trials it has been shown to be more effective and less toxic than Amp B weekly at 1 mg/kg.\[57\] Oral itraconazole at 200 mg/day successfully prevented relapse of cryptococcal meningitis in 34 of 39 patients with AIDS.\[64\]

Before the era of highly active antiretroviral therapy (HAART), prophylaxis for cryptococcal meningitis with fluconazole was life long. With a promise of better recovery in immune function, discontinuation of therapy safely in those with sustained (>3 months) undetectable viral loads and a CD4 count >100 cells/ml has been suggested. In a retrospective review of pa-
tients, who had discontinued secondary fluconazole prophylaxis among HIV-infected patients on HAART, 4 of 100 patients redeveloped cryptococcal meningitis within 28 months. In three of these patients, the serum cryptococcal antigen became positive at relapse.\[50\]

Prognosis

Amphotericin B has improved the prognosis in cryptococcal meningitis, an infection that was uniformly lethal earlier. With therapy, less than a quarter of patients die. Poor prognostic factors in cryptococcal meningitis include: presence of visual disturbances, hyponatremia, an initial positive India ink for the pathogen, high-CSF opening pressure, low-CSF glucose, and low-CSF leucocytes (<20 cells/ml), cryptococci seen in extra neural sites, absence of cryptococcal antibody with a CSF cryptococcal antigen titer ≥1:32, and associated corticosteroid therapy or lymphoreticular malignancy. These risk factors emphasize the study of the quantity of the yeasts and the deficiency in host responses. Follow up includes monitoring decreasing headache as a symptom, recovery of a normal mental status, and CSF leucocyte count 20 cells/ml, all of which are favorable. In spite of good therapy, the life expectancy in HIV-infected patients with CNS cryptococcosis is about 6 months after diagnosis, and this could be improved by use of HAART.

Prevention of cryptococcosis

Most cryptococcal infections occur in patients with a CD4 cell count <200 cells/ml. Prophylaxis with fluconazole for oral candidiasis prevents oral thrush, nail and cutaneous fungal infections, cryptococceria, and fungal colonization. The role of antifungal therapy, especially fluconazole, for primary prevention of cryptococcal meningitis is unclear, and as of now, it is not recommended due to concerns on the risk of inducing resistance as documented in Cambodia.\[63\] In a small study from Thailand, the use of fluconazole as primary prophylaxis in patients with a CD4 less than 100 cells/ml showed that patients in the placebo arm had a 2.2-fold higher risk of developing cryptococcal meningitis and a 4.3-fold increased risk of dying.\[59\] Another study from Thailand has also shown that itraconazole prophylaxis at 200 mg/day significantly reduced the risk of invasive fungal infections – cryptococcosis and P. marneffei in those with advanced HIV disease (CD4 less than 100 cells/ml) but this did not result in improved survival.\[60\] Further studies are required to clarify the issue.

Data from Indian studies

Data from our institution published earlier had suggested a 7% occurrence (4 of 61 patients) of cryptococcal meningitis as the AIDS defining condition at presentation.\[61\] In another study evaluating the etiology of prolonged fever in HIV-infected adults, cryptococcosis was detected to be the causative agent in 10% of these patients.\[62\] Cryptococcal meningitis was reported in 2.5% (12 of 483) of HIV-infected patients from a tertiary institution with a mortality of 50%.\[63\]

In a 5-year retrospective cohort and 1-year prospective cohort study (total 105 patients) at our center (2000–2001), we assessed clinical profile, outcomes, and poor prognostic markers in patients with cryptococcal meningitis. More than 75% of them were <40 years of age (mean 35.4 years), as did other studies.\[64,65\] Males were predominant (90%). The common manifestations at presentation were headache (88%), fever (73%), vomiting (57%), and weight and appetite loss (49%). Seizures and focal neurological deficits were rare (<1%). Prior AIDS defining disease, predominantly TB was seen in 55% of the patients. Signs of meningeal affection occurred seen in 73% and the mean Glasgow coma scale at admission was 14, suggesting normal mentation at admission. In a study from Chandigarh, headache and fever were the commonest manifestations, seen in a majority of the patients and signs of meningeal irritation were noted in less than half the patients (7 of 15). Focal deficits were noted only in 20% of the patients (3 of 15).\[64\]

In our study, where fungal cultures and cryptococcal antigen were positive in all patients and 83% of patients were India-ink positive, the mean CSF WBC count was 107 cells/ml, protein 88.6 mg%, and glucose 48.6 mg%. In a similar study from another tertiary institution, CSF cells was <20 cells/ml in half the patient tested (9 of 18) and India ink positivity 72% and cryptococcal antigen being detected in all patients.\[65\]

In our study, the variables found to be independently associated with poor outcome were a low-CSF protein, an absolute blood lymphocyte count <1000 cells/ml and a CSF WBC count <20 cells/ml. On post-treatment follow up, 45% of our patients died within 3 months, and one-sixth of them could not be traced, giving a 3-month survival rate of 40%. Similar survival rates of 40% (6 of 15)\[64\] and 44% (7 of 15)\[65\] were seen at other centers.

Cryptococcosis is a sentinel infection that signals the perturbation of the host’s immune status. Newer and safer antifungal agents allowing longer initial treatment and effective suppressive regimen is needed along with interventions to improve host’s immune response to combat C. neoformans are needed.

References


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