Review Article

Cryptococcal Infection

Woraphot Tantisiriwat, M.D., MPH*
William G. Powderly, M.D., FRCPI**

ABSTRACT

Prior to the 1980s, cryptococcal infection was rare. Most infections occur in patients with impaired cell-mediated immunity. However, since the beginning of the acquired immunodeficiency syndrome (AIDS) epidemic, the infection has emerged as a major cause of morbidity and mortality in patients infected with the human immunodeficiency virus (HIV) who have CD4 cell count of less than 200 cells/mm^3. With the use of highly active antiretroviral therapy (HAART) for the treatment of HIV infection and the widespread use of the azole antifungals, the incidence of cryptococcal infection has declined in the Western world. However, it remains extremely important in developing countries. This article reviews current aspect in microbiology, epidemiology, pathogenesis, clinical manifestations and treatment of crytococcal infection. (*J Infect Dis Antimicrob Agents 2004;21:29-40.*)

INTRODUCTION

Prior to the 1980s infection with the fungus *Cryptococcus neoformans* was rare, occurring mainly among persons with impaired cell-mediated immunity[1]. Up to half of the cases of cryptococcal disease were associated with lymphomas, and many other patients with cryptococcosis received corticosteroid therapy before the onset of the infection. However, since the beginning of the acquired immunodeficiency syndrome (AIDS) epidemic, cryptococcosis has emerged as a major cause of morbidity and mortality in persons infected with the human immunodeficiency virus (HIV) who have CD4 cell count of less than 200 cell/mm^3.[2-3] Disseminated cryptococcosis has been the most common life threatening fungal infection in patients with AIDS, affecting up to 8 percent of patients with advanced HIV infection.[2-3] With the use of highly active antiretroviral therapy (HAART) for the treatment of HIV infection and the widespread use of the azole antifungals, the incidence of invasive cryptococcosis in HIV-infected population has declined[4] in the Western world. However, cryptococcosis remains extremely important in other parts of the world. Cryptococcal meningitis is the second most common opportunistic infection (after tuberculosis) associated with AIDS in sub-Saharan Africa and South Asia. This article reviews current aspect in microbiology, epidemiology, pathogenesis,

*Clinical Instructor, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand.

**Division of Infectious Diseases, Department of Medicine, Washington University School of Medicine, 660 South Euclid Ave., Campus Box 8051, St. Louis, MO 63110, USA.

Email: wpowderl@imgate.wustl.edu

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Reprint request: Woraphot Tantisiriwat, M.D., MPH, Clinical Instructor, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand.

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clinical manifestations and treatment of cryptococcal infection.

Microbiology and Epidemiology

Cryptococcus neoformans is the only known species of all of the cryptococcal species to be pathogenic.[5] It is an encapsulated, round-to-oval-shape yeast form that reproduces by budding. It has a surrounding polysaccharide capsule ranging from 1 to over 30 μm when cultivated in the laboratory.[6] In nature, it is smaller and poorly encapsulated. Mycelia are produced bearing basidiospores ranging from 1 to 8 μm in its perfect state, *Filobasidiella neoformans*. *Filobasidiella neoformans* has never been isolated from patients or in nature[7].

Cryptococcus neoformans can be distinguished into four serotypes according to their polysaccharide capsule, serotype A and D are classified as variety *neoformans* and serotype B and C are classified as variety *gattii*.[1] These two pathogenic varieties can be distinguished by growth characteristics on canavanine-glycine-brothymol blue agar.[8]

Cryptococcus neoformans var. *neoformans* is seen worldwide and has been associated with pigeon and other bird droppings and soils contaminated with these droppings. It usually affects the immuno-compromised host.[9] *Cryptococcus neoformans* var. *gattii* is endemic in tropical and subtropical areas such as Australia and Southern California in where it is associated with flowering of the river red gum tree (*Eucalyptus camaldulensis*).[10] It affects mainly healthy individuals[9] and is rare in HIV-infected patients.

Pathogenesis

Initial infection is postulated from inhalation of basidiospores or encapsulated forms leading to colonization of the airways and subsequent infection.[1,11] Pulmonary macrophages are critical in host control of the yeast inoculum[12], and complement-mediated phagocytosis appears to be the primary initial defense against cryptococcal invasion.[6] Other interactions associated with CD4+ and CD8+ T cells, as well as cytokines are also important.[12-15] The absence of an intact cell-mediated immune response results in ineffective ingestion and killing of the organism leading to dissemination and increased cryptococcal burdens. The role of humoral immunity in control of cryptococcal infection is controversial. *In vitro* studies of antibodies to the soluble capsular polysaccharide of *Cryptococcus neoformans* reveal enhanced phagocytosis, increased fungicidal activity of leukocytes, and increased fungistatic activity of natural killer cells.[16-19] Animal models of both polyclonal and monoclonal antibody immunization have had varying results.[20-23]

Factors associated with virulence in *Cryptococcus neoformans* include its polysaccharide capsule, melanin production, the mating type, and growth at 37 C (thermotolerance).[24-26] The capsule, composed mainly of glucuronoxylomannan, was the first virulence factor associated with the disease.[27]

Cryptococcus neoformans is distinguished from other yeasts by its ability to assimilate urea and its possession of membrane-bound phenoloxidase enzymes, which are able to convert phenolic compounds into melanin. *Cryptococcus neoformans* strains which produce melanin are more virulent in mouse models than strains which do not produce melanin.[18-19] In addition, murine cells with melanin appear more resistant to phagocytosis.[25] It is postulated that the propensity of *Cryptococcus neoformans* to invade the central nervous system (CNS) may be due to its ability to synthesize melanin from catecholamines, which are present in large concentration there.[28]

Presentations and Diagnosis

The most common manifestation of cryptococcal infection is meningitis.[29] Most patients develop insidious features of subacute meningitis or meningoencephalitis, with fever, malaise, and headache, and are generally symptomatic for at least 2 to 4 weeks before presentation. In patients with a more subacute or chronic course, mental status changes such as forgetfulness and coma can also be seen. Classic meningeal symptoms and signs such as stiff neck and photophobia occur in

only about a-quarter-to-a-third of all patients and generally are less likely to occur in HIV-positive patients.[30] The typical pattern in the cerebrospinal fluid (CSF) is chronic meningitis with a lymphocytic pleocytosis. However, the CSF may appear normal in HIV-positive patients with cryptococcal meningitis, since the usual response to infection is usually markedly blunted.[29,31] In fact, fewer than half of HIV-positive patients with cryptococcal meningitis have an elevated protein level, only about one-third have hypoglycorrhachia, and only about 20 percent have more than 20 white blood cells per cubic millimeter of CSF.[31] The opening pressure is usually elevated in patients with cryptococcal meningitis (up to 70 percent of patients present with pressures greater than 20 cm H_2O) and is an important issue associated with therapy.[32] India ink stain of the CSF is positive, showing encapsulated yeasts, in about 75 percent of cases, and the cryptococcal antigen titer in the CSF is almost invariably positive with the sensitivity of 93-100 percent and specificity of 93-98 percent.[33] Serum cryptococcal antigen (sCRAG) is usually elevated in 95 percent of patients with meningitis.[33-34] A positive of sCRAG with titer above 1:8 suggests disseminated cryptococcosis.[35] Such patients should be evaluated for possible meningeal involvement. There is little value in the serial measurement of CSF and sCRAG in the routine management of cryptococcal meningitis.[36-37] CSF cryptococcal antigen titers should decrease after effective therapy. There is no evidence to support following serial serum antigen, which shows no correlation with outcome of antifungal therapy. Management should rely on clinical assessment not on the cryptococcal antigen alone.

False positive of sCRAG can be observed in infection with *Trichosporon beigelii*, or due to residual disinfectant on laboratory test slides and inactivated pronase in a test kit.[38-40] Culture of *Cryptococcus neoformans* from any body site should also be regarded as an indication for further evaluation and initiation of therapy. However, colonization of *Cryptococcus* can be found in respiratory system and therapy might not be necessary in immunocompetent patients with no symptoms and negative sCRAG.[41]

Cryptococcus neoformans can invades sites other than the meninges. Isolated pulmonary disease has been well described.[9,41] It usually presents as a solitary nodule in the absence of other symptoms. *Cryptococcus* pneumonia has also been described.[9, 41-42] In immunocompromised patients, especially those with AIDS, disseminated disease is common.[30, 43] About half of HIV-positive patients with cryptococcal meningitis have evidence of pulmonary involvement at presentation, with clinical symptoms such as cough or dyspnea and abnormal chest radiographs. The chest radiographic finding is usually diffuse interstitial infiltrates in immunocompromised patients or focal lesions in immunocompetent patients. Concomitant opportunistic infections, especially with *Pneumocystis jirovecii*, occur in about 15 to 35 percent of patients.[44,45] Cutaneous involvement is common and suggests disseminated disease.[46,47] The most common skin involvement resembles that of molluscum contagiosum.[48] As many as three-quarters of patients with cryptococcal meningitis have positive blood cultures.[49] Infection of the bone, eye, heart, adrenal glands, prostate and urinary tract has also been described.[50-57] The prostate gland can act as a reservoir of infection and potential source of reinfection after completion of therapy.[56-57]

Therapy

Management of cryptococcal infection depends on the extent of the disease especially to which organ involved and the immune status of the patient. A solitary pulmonary nodule in a normal host may not need treatment, provided that the patient has careful follow-up.[42] The advent of relatively safe antifungals such as fluconazole prompts a short course of therapy for most patients with localized disease. Extrapulmonary disease is generally managed in the same way as meningitis. A search for the underlying problems should be initiated in patients who are not known to be immunosuppressed including an HIV

antibody test and CD4 lymphocyte count, as cryptococcal infections have been described as one of manifestations of so-called isolated CD4 T cell lymphocytopenia.[58-59] Drugs generally used in the treatment of cryptococcal infection are summarized in Table 1.[60]

Table 1. Drugs used in the treatment of cryptococcal infection.

Drugs	Dosage	Side Effects	Drug Interactions	Comments
Amphotericin B	0.7-1.0 mg/kg/d 3-6 mg/kg/d (liposomal) 5 mg/kg/d (lipid complex)	Immediate hypersensitivity reaction, fever, hypotension, nausea and vomiting during administration, hypokalemia, and nephrotoxicity.	Nephrotoxic drugs (e.g. aminoglycosides, pentamidine, foscarnet, cidofovir).	Liposomal or lipid complex formulation should be considered in patients with renal dysfunction.
Flucytosine(5-FC)	25 mg/kg q 6 hr	Gastrointestinal symptoms, bone marrow suppression.	Nephrotoxic drugs.	Dosage must be reduced in patients with renal dysfunction. Drug levels should be monitored.
Fluconazole	400 mg/d (acute therapy) 200 mg/d (suppressive therapy)	Nausea, rash, and hepatitis.	Rifabutin (increased rifabutin levels); rifampin (decreased fluconazole levels).	Dosage may need to be adjusted in renal dysfunction.
Itraconazole	200-400 mg bid	Nausea, abdominal pain, rash, headache, edema, and hypokalemia.	Rifamycins, ritonavir, phenobarbitol, phenytoin all decrease itraconazole levels. The effect of nevirapine is unknown. The drug should not be used concomitantly with terfenadine or astemizole. Antacids, histamine-2 blockers decrease itraconazole absorption. Itraconazole itself acts as a moderate inhibitor of cytochrome P-450 system and can increase levels of indinavir, cyclosporin, digoxin, and phenytoin.	Absorption of itraconazole is dependent on food and gastric acid and may be erratic. The newer solution is better absorbed.

Non-HIV-infected persons

For non-HIV-infected persons with cryptococcal meningitis, with no evident risk factors for a poor outcome (shown in Table 2 and Table 3), 4-week course of combination therapy of 0.3 mg per kg of amphotericin B plus 37.5 mg per kg of 5-FC is comparable of 6-week course. Without 5-FC, 10-week course of 0.4 mg/kg of amphotericin B is recommended.[60-62] However, based on studies for HIV-infected patients, the preference dosage of amphotericin B is 0.7 mg/kg.[63] Although it is unclear whether 5-FC is necessary with higher doses of amphotericin B. Azole therapy (i.e. fluconazole 400 mg PO daily) for 3-6 months is less likely to be effective in such patients but has not been well studied.[64-65] In mild-to-moderate pulmonary cryptococcosis without meningeal involvement, it is recommended to treat initially with fluconazole. Itraconazole or amphotericin B alone is alternatives.[66-67] Fluconazole can be used to treat patients (or complete a course of treatment) who can not tolerate amphotericin B or patients without neural involvement. For meningitis, there is no controlled trial of fluconazole especially comparing initial azole therapy with an amphotericin B-based regimen. Immunocompetent patients do not require any long-term suppressive therapy.

Management of cryptococcal meningitis in the setting of organ transplantation or lymphoma usually requires more prolonged therapy.[67] It is recommended to use the same regimen for AIDS patients with cryptococcal infection in this setting, i.e. an initial period of treatment using amphotericin B plus 5-flucytosine followed by fluconazole. An area of considerable uncertainty is the duration of fluconazole therapy after acute therapy. In general, it is recommended to continue suppressive antifungal therapy for at least six months to one year after the completion of acute treatment.[67] For patients receiving long-term corticosteroids, reduction of the dosage to 10 mg/d, if possible, may result in improved outcome to antifungal therapy.[67]

The current guidelines of the Infectious Disease Society of America for treatment of cryptococcal meningitis with AIDS recommend initial amphotericin B therapy (with 5-FC if possible) which is followed by consolidation with azole therapy. It should be recognized however that the outcomes in clinical trials may be better than those observed in clinical practice. A recent study from Thailand using such an approach reported a mortality rate of 16 percent and 24 percent at 2 and 4 weeks.

Table 2. Factors predicting relapse of cryptococcal meningitis.

Immunosuppression
Presentation with neurologic abnormalities
CSF leukocyte count less than 20 cells per cubic millimeter
CSF antigen titer greater than 1:32
Positive india ink stain after 4 weeks of treatment
CSF antigen titer greater than 1:8 after 4 weeks of treatment

Table 3. Factors at baseline predictive of a poor outcome in AIDS patients with cryptococcal meningitis.

Decreased mental status at diagnosis
CSF leukocyte count less than 20 cells per cubic millimeter
High titer of CSF cryptococcal antigen
Positive blood culture for *C. neoformans*
Age less than 35 years
Hyponatremia

The availability of the alternative formulations of amphotericin B raises the issue of their use in cryptococcal meningitis. A randomized study comparing amphotericin B lipid complex with amphotericin B deoxycholate in 55 HIV-positive patients with cryptococcal meningitis noted less hematologic and nephrotoxicity in the lipid complex arm.[68] There was a trend favoring "standard" amphotericn B in mycologic outcome. Small randomized studies of liposomal amphotericin B (4 mg/kg) compared with conventional amphotericin B (0.7 mg/kg) noted an earlier CSF sterilization rate in patients in the liposomal preparation arm with also less

nephrotoxicity.[69-70] Thus, the role of lipid preparations of amphotericin B remains uncertain, although they may be useful in patients with impaired renal function.

HIV-infected persons

For treatment of cryptococcal meningitis in HIV-infected patients, the current guideline recommends initial 2-week therapy of amphotericin B (with 5-FC if possible) which is followed by 8-10 weeks of consolidation fluconazole therapy. The availability of the alternative formulations of amphotericin B raises the issue of their use in cryptococcal meningitis. However, the role of lipid preparations of amphotericin B remains uncertain, although they may be useful in patients with impaired renal function.[68-70] There remains some interest in oral combination therapy i.e. fluconazole with 5-FC, itraconazole with 5-FC.[71-73] Nevertheless, further studies should determine the value of these combination therapies.

A very important aspect of management of cryptococcal meningitis in AIDS is the recognition that the clinical deterioration may be due to increased intracranial pressure (ICP) and that this aspect may not respond rapidly to antifungal therapy.[74-75] There were studies which showed a relationship between baseline opening pressure and long-term outcome, with the median survival in patients with the highest pressures being significantly less than that in patients with normal pressure.[76] Hence, all patients with cryptococcal meningitis should have opening pressure measured when a lumbar puncture is performed and strong consideration should be given to reducing such pressure if the opening pressure is high (more than 20 cm H_2O). Daily lumbar puncture for removal of spinal fluid is recommended to reduce opening pressure to less than 20 cm H_2O or 50 percent of the initial opening pressure. If the elevated opening pressure is still persisted with neurological symptoms despite serial lumbar puncture, a continuous lumbar drainage should be considered. In case of failure to control pressure with a continuous lumbar drain, a lumboperitoneal shunt is required.[77] Acetazolamide has been suggested for treatment of increased ICP, but a randomized trial was prematurely discontinued because of more side-effects with acetazolamide.[78] At present, the role of corticosteriods in this situation is not known so it is not routinely recommended.

Lifelong maintenance therapy is required in AIDS patients with cryptococcal infection to prevent relapse of infection, if antiretroviral therapy is not provided or is ineffective. Fluconazole 200 mg daily is the drug of choice.[79-80]

For primary prophylaxis in advanced HIV disease, fluconazole 200 mg daily also prevents cryptococcal meningitis in patients with AIDS. Fluconazole 400 mg weekly has equal effect in prevention of cryptococcal infection but is less effective for oral candidiasis compared with daily fluconazole.[81] Itraconazole 200 mg daily was also effective in preventing cryptococcosis.[82] In spite of the clear evidence of protective effect, the overall utility of primary prophylaxis is unclear. The guidelines from the US Public Health Service and Infectious Disease Society of America for prophylaxis in patients with HIV infection do not endorse the routine use of primary antifungal prophylaxis.[83] However, the situation could be quite different in the developing world, especially where cryptococcal or other fungal infection is highly prevalent. Preliminary data from a small randomized trial of primary prophylaxis with fluconazole (400 mg once weekly) in Thailand showed no difference in the incidence of fungal infections but a survival difference that was marginally significant.[84] This is a preliminary study, and it is too small to instill confidence that the finding is real, but this observation supports large randomized trials of primary prophylaxis in areas such as South and Southeast Asia and sub-Saharan Africa, where the incidence of cryptococcal and other endemic fungal infection is very high.

One issue that may arise when starting anti-retroviral therapy in patients with cryptococcal infection is immune reconstitution illness. Immune reconstitution illness is a newly recognized syndrome, the onset of which is usually within the first 3 months

of starting antiretroviral therapy.[85] It is thought to represent an inflammatory state induced by the newly restored immune system against pathogens that have previously infected the HIV-positive host. It has been well described with mycobacterial infection and has also been seen with cytomegalovirus (CMV) retinitis, hepatitis C and hepatitis B virus infections, fungal infections (cryptococcosis and histoplasmosis), *Herpes zoster*, progressive multifocal leukoencephalopathy (PML), and even malignant and noninfectious disorders.[85]

Immune reconstitution associated with cryptococcosis can be manifested with highly unusual localizations, and a varying spectrum of clinical findings.[86-91] Patients may present with cryptococcal lymphadenitis, with a recurrence of meningitis that is characterized by a CSF leucocytosis and negative cultures, or by the appearance of cryptococcomas. Management should include continuation of antifungals and the antiretroviral therapy, probably with administration of corticosteroids or NSAIDs for symptomatic control. The common factor in these cases is the apparent enhancement of cell-mediated immunity, which paradoxically worsens the clinical course in a response to a burden of fungal antigens, and does not represent the progression of infection or the failure of antifungal therapy.

With the use of potent antiretroviral therapy for AIDS, it has also been observed in many case series that secondary prophylaxis for many opportunistic infections can be discontinued safely in patients who have sustained immunologic response on effective antiretroviral therapy.[92] Accumulating data indicates that it is also safe to discontinue secondary prophylaxis in patients with a history of disseminated fungal infection who have had sustained immunologic responses. There have been several case series and one prospective trial which demonstrated the safety of discontinuing secondary prophylaxis in patients with prior cryptococcal meningitis.[93-97] In light of these data, the current USPHS/IDSA guidelines recommend that it may be reasonable to discontinue secondary prophylaxis for cryptococcal meningitis in patients who have had sustained immunologic responses.

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