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# Cryptococcal meningitis

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Cryptococcal meningitis is a common opportunistic infection in AIDS patients, particularly in Southeast Asia and Africa. Cases also occur in patients with other forms of immunosupression and in apparently immunocompetent individuals. Mortality from HIV-associated cryptococcal meningitis remains high (10–30%), even in developed countries, because of the inadequacy of current antifungal drugs and the complication of raised intracranial pressure. In cohorts of HIV-infected patients from sub-Saharan Africa, cryptococcosis has accounted for 13–44% of all deaths. Optimal current therapy is with amphotericin B 0.7–1 mg/kg/day plus flucytosine 100 mg/kg/day for 2 weeks, followed by fluconazole 400 mg/day for 8 weeks and 200 mg/day thereafter. Saline loading reduces amphotericin B nephrotoxicity. If there is no contraindication on CT head scan, repeat lumbar puncture with drainage of cerebrospinal fluid (CSF) is recommended for patients with very raised CSF opening pressure. Expansion of antiretroviral programmes raises the prospect of transforming the long-term prognosis of these patients, provided that they survive the acute phase of the illness. Studies are needed to define more fungicidal drug regimens and to improve the treatment of raised intracranial pressure.



## Introduction

The incidence of infections caused by the encapsulated yeast *Cryptococcus neoformans* has risen markedly over the past 20 years as a result of the HIV epidemic and increasing use of immunosuppressive therapies.[1] 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.[2,3] 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*.[4,5] Mortality from HIV-associated cryptococcal meningitis remains high (10–30%), even in developed countries, because of the inadequacy of current antifungal

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drugs and combinations, and the complication of raised intracranial pressure.[6,7] In the developing world, patients tend to present later, flucytosine is often not available and there may not be the resources or facilities for inpatient intravenous therapy with amphotericin B. The result of this combination of factors is that in cohorts of HIV-infected patients from sub-Saharan Africa, cryptococcosis has accounted for 13–44% of all deaths.[8–10]. However, the expansion of antiretroviral programmes now raises the prospect of transforming the long-term prognosis of these patients, provided that they survive the acute phase of the illness. Therefore there is an urgent need to define more fungicidal drug regimens, and to improve the understanding and treatment of raised intracranial pressure in this setting.

## Mycology, ecology and epidemiology

*Cryptococcus neoformans* is an environmental saprophyte. The rarity of its isolation as a human commensal and of human-to-human transmission suggests that human infection is an accidental dead-end event in its life-cycle. However, uniquely amongst the thousands of basidiomycete fungi, *C.neoformans*, under selective pressure in the environment, has evolved a number of characteristics that, perhaps by chance, also enable it to survive within human and other mammalian and avian hosts.[11] Sexual reproduction between a- and α-mating type isolates of *C.neoformans* has been described in the laboratory, with the formation of hyphae and, following meiosis, basidiospores, but in the environment and in clinical specimens *C.neoformans* is found as a budding yeast. The yeasts are spherical to oval cells, 5–10 μm in diameter, with a polysaccharide capsule which is a major virulence factor and the substrate detected by cryptococcal antigen tests.

Two varieties and five serotypes of *C.neoformans* were recognized, but the varieties have now been accorded species status: var. *neoformans*, now *C.neoformans* (serotype A, D and AD; based on capsular polysaccharide antigens), and var. *gattii*, now *C.gattii (*serotypes B and C). Most clinical isolates are serotype A. *Cryptococcus neoformans* is found worldwide in association with soil contaminated with bird, particularly pigeon, excreta and usually causes infection in immunosuppressed individuals. *Cryptococcus gattii*, on the other hand, is found primarily in tropical and subtropical regions, has been associated with several species of eucalyptus trees and causes infection predominantly in apparently immunocompetent individuals. In addition, studies from South America have reported both species in the decaying heartwood of a number of trees.[12] That this may be an important ecological niche is suggested by the presence of a laccase enzyme in both species that could be involved

in the breakdown of lignin and the the fact that *C.neoformans* and *C.gattii* are closely related to other wood-rotting fungi.[13] Serological studies suggest that most individuals are exposed to the organism, starting after the first 2 years of life.[14] However, the precise circumstances and frequency of exposure, like the ecology of the organism, are still not completely understood. Emphasizing this point, for unclear reasons, an unprecendented outbreak of *C.gattii* infections has occurred since 1999 in over 50 immunocompetent patients and animals as diverse as dogs, cats, llamas and porpoises on Vancouver Island, Canada.[15]

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 5–10% 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 7 cases per 1000 in 2000.[16]

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.[3] 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.[8] It seems most likely the high incidence of cryptococcosis in parts of Africa and Asia reflects differences in exposure rather than host susceptibility or cryptococcal strain virulence, although no studies have addressed this issue. As a consequence of the increase in HIV-associated cryptococcosis, there has been a shift in the epidemiology of meningitis; cryptococcal meningitis is now the leading cause of community-acquired meningitis, ahead of tuberculous and bacterial meningitis, accounting for 20–45% of laboratory-confirmed cases of meningitis in Southern Africa.[4,5]

Other groups at risk of cryptococcosis are patients with sarcoidosis, lymphoproliferative disorders and those on immunosuppressive therapy. In a series of 306 HIV-negative patients with cryptococcosis, the predisposing conditions were steroids (28%), organ transplant (18%), chronic organ failure (liver, lung, kidney) (18%), malignancy (18%) and rheumatological disease (13%).[17] Twenty-two per cent of patients had no identified predisposing factor. Infection in apparently immunocompetent patients is also a feature of *C.gattii* cryptococcosis in Australia and Southeast Asia. Increasing use of organ transplantation and developments in immunosuppressive therapies for cancer and other systemic diseases since the 1970s has led to an increase in the incidence in non-HIV-associated cryptococcosis in the USA to up to 1 case per 100 000 persons per year in the 1990s.[18]

## Pathogenesis and host defence

Infection is probably acquired by the inhalation of small yeast cells or, possibly, basidiospores. The primary pulmonary infection is frequently asymptomatic and may be eradicated or contained within granulomata. However, depending on host factors, inoculum, and possibly isolate virulence, the organism may disseminate either acutely or after a period of latency to extrapulmonary sites, with a particular predilection for the brain.

Direct evidence for latent infection was provided by autopsy studies demonstrating cryptococcal cells within pulmonary granulomata in individuals dying of unrelated causes.[19] Goldman *et al.*[20] have 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. Work by Dromer and colleagues,[21] who typed isolates from patients diagnosed with cryptococcosis in France, some of whom were from Africa but had lived in France for a median of >9 years, suggested that reactivation of such latent infection may be important in HIV-associated cryptococcosis. There was a significant clustering of isolates from African compared with European patients, suggesting that patients had acquired their isolates long before the development of clinical disease.

Much has been learnt about the immune response to cryptococcal infection from study of animal models and from *in vitro* experimentation. 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 necrosi 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,[22] and the cryptococcal mannoproteins that are important in stimulating specific T-cell immunity.[23] In addition, we have now directly correlated immune parameters at the site of infection in the cerebrospinal fluid (CSF) with survival and the rate of clearance of infection determined by serial quantitative cultures of CSF over the first 2 weeks of therapy.[24] The trio of pro-inflammatory cytokines TNF-α, IFN-γ, and IL-6 were shown to be associated with survival. In a multivariate analysis, only antifungal treatment group and baseline CSF IFN-γ levels were

independently associated with the rate of clearance of infection, confirming the importance of quantitative differences in IFN-γ in the control of cryptococcal infection in the human system (Fig. 1).[24] The possible role of polymorphisms in pro-inflammatory cytokine genes in predisposition to and severity of cryptococcal disease is currently being studied.

*Cryptococcus neoformans* has a number of virulence factors that enable it to survive and replicate in the human host,[11] especially if specific T-cell immunity is compromised: the ability to grow at 37°C, the capsule, which is antiphagocytic and down-regulates cellular and humoral immune responses when shed into host tissues, and laccase and melanin that interfere with oxidative killing by phagocytes. Production of melanin from l-dopa by the enzyme laccase may account for the predilection of the organism for the central nervous system (CNS). *Cryptococcus neoformans* is an intracellular as well as an extracellular pathogen, and can survive and replicate within acidic macrophage phagolysosomes,[25] depending on the origin and state of activation of the cells. Following intracellular replication, capsule polysaccharide accumulates in vesicles, leading to permeabilization of the phagolysosomal membrane and cytotoxicity.[11] Interestingly, the ability of *C.neoformans* to survive in macrophages may have evolved, as in *Legionella pneumophila*, from strategies to survive in predatory environmental amoebae.[11]

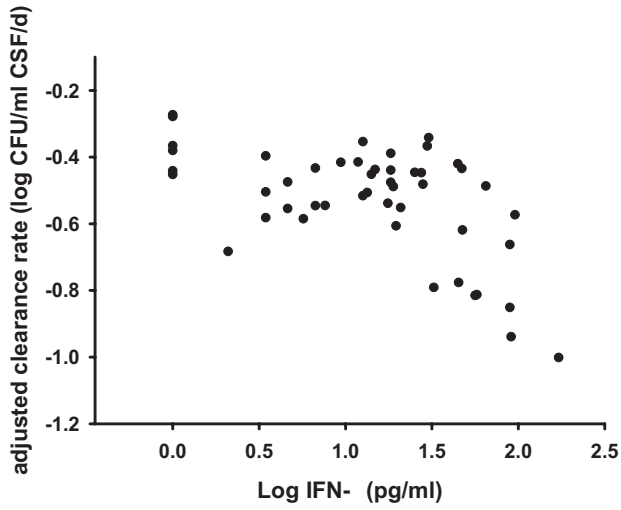


**Fig. 1** Association between CSF IFN-γ levels and rate of clearance of infection in cryptococcal meningitis (from reference 24, with permission). The rate of decrease in log CFU/ml CSF/day has been adjusted for the effect of treatment group, according to the results of a linear regression model including treatment group and log IFN-γ. Log IFN-γ was independently associated with a more rapid fall in CFU (increase in rate of fall in CFU for each increment in log IFN-γ = 0.15 log CFU/ml CSF/day (95%CI, 0.08–0.22; $P < 0.001$). Reproduced with permission from A. Siddiqui *et al.* (2005) *J. Immunol*, 174, 1746–1750. Copyright 2005 The American Association of Immunologists Inc.

An autopsy series describing the neuropathology of 27 patients with cryptococcal meningoencephalitis found that, in contrast with non-HIV-associated cases, AIDS patients had no granulomata, were more likely to have involvement of brain parenchyma in addition to meningitis and had greater numbers of yeast cells which tended to be extra- rather than intracellular.[26] These findings support the role of deficient cell-mediated immunity, notably altered macrophage and microglial function, in determining the distinct pathology of cryptococcal meningoencephalitis in AIDS.

## Clinical presentation

*Cryptococcus neoformans* can infect any organ in the body, but has a predilection for the lung and the CNS. The lung is the usual portal of entry and symptoms range from asymptomatic colonization to severe pneumonia.

Meningitis is the most frequent manifestation of cryptococcosis. Infection of the subarachnoid space is accompanied by involvement of the brain parenchyma, and therefore the term meningoencephalitis may be more appropriate. Cryptococcal meningitis should always be included in the differential diagnosis of chronic or subacute meningoencephalitis, since clinical features are not specific. Patients usually present with headache, fever, malaise and altered mental status over several weeks. Signs are often absent, but may include meningism, papilloedema, cranial nerve palsies and other focal neurological deficit, and depressed conscious level. Complications are common; raised intracranial pressure (see below) in the absence of ventricular dilatation may cause profound visual or hearing loss. Less commonly, patients may develop cognitive impairment and gait ataxia due to obstructive hydrocephalus with ventricular dilatation.

In HIV-infected patients, the disease is associated with profound immunosuppression, usually occurring at CD4 counts <100 cells/μl. There is a greater likelihood of involvement outside the CNS and relapse if antifungal therapy is discontinued prior to effective antiretroviral therapy. Compared with HIV-negative patients, the presentation tends to be more acute, and is associated with higher serum cryptococcal antigen titres and a poor CSF inflammatory response (white blood cell count <20/μl).

*Cryptococcus gattii* causes infection in apparently immunocompetent patients in tropical areas, especially Northern Australia and Papua New Guinea. Focal pulmonary and CNS mass lesions tend to be more common, the duration of symptoms longer and fever less common in this group.[27]

## 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. There is no pathognomonic brain image of cryptococcal meningitis. CT scans may be normal or reveal meningeal enhancement, single or multiple nodules (cryptococcomas), cerebral oedema, or hydrocephalus. 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

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. On the other hand, in non-HIV-associated disease, especially in apparently immunocompetent patients, cultures and antigen tests of CSF may sometimes be negative and the diagnosis is hard to exclude.[28] Large-volume CSF cultures and repeated lumbar punctures may be needed in this setting. The usual precautions apply regarding lumbar puncture in this setting, and a CT head scan prior to lumbar puncture would always be preferable in suspected cryptococcal meningitis. However, this is not possible in many areas of high incidence, and it should not delay diagnosis.

### *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. CSF protein is usually elevated and CSF glucose may be low. Indian ink examination is positive in 70–90% of AIDS patients but in only ~50% of non-AIDS patients.

### *Culture*

*Cryptococcus neoformans* from CSF, blood or other sites produces white mucoid (depending on the capsule thickness) colonies, usually within 48–72 h, on most bacterial and fungal (Sabouraud dextrose agar)

media. Although *C.neoformans* grows at 37°C, a temperature of 30–35°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. 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.

### *Serology*

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.[29] 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.[30]

### *Histology*

In tissue sections, cryptococcal cells, in common with most fungi, are positive with Gomori methenamine silver and periodic acid–Schiff staining. Mucicarmine, which stains the capsule red, and Fontana–Masson, which stains fungal melanin reddish-brown, are more specific for the organism.

## Treatment

### *Antifungal drugs*

Untreated cryptococcal meningitis is uniformly fatal, although survival can range from years in those without apparent immunocompromise to only a few weeks in HIV-associated infection.[31]

Amphotericin B, a polyene introduced in the 1950s, is a fungicidal agent that binds to ergosterol in the fungal plasma membrane, increasing permeability to protons and monovalent cations such as potassium. Antifungal activity may also be due to stimulation of the generation of

reactive forms of oxygen in fungal as well as immune cells,[32] and stimulation of pro-inflammatory cytokine production.[33] Amphotericin B was the first effective therapy and, at doses of 0.3–0.5 mg/kg/day for 10 weeks, led to cure rates of over 50% in non-HIV-associated infection. It has concentration-dependent activity,[34] and more recent trials at doses of 0.7 mg/kg/day have yielded improved results[6]. Current guidelines recommend doses of 0.7–1 mg/kg/day, although there are no clinical data comparing 0.7 mg/kg with 1 mg/kg.[35] Nephrotoxicity may be a problem, necessitating careful monitoring. However, nephrotoxicity is reduced by fluid and saline loading (1 l/day normal saline, unless contraindicated),[36] and in this patient group amphotericin B is generally well tolerated for 2 weeks.[6,37] Alternative lipid formulations, of which liposomal amphotericin B is the best studied, allow the delivery of much larger doses of amphotericin B and appear to be at least as effective and less nephrotoxic than conventional amphotericin B deoxycholate. In a small randomized study, liposomal amphotericin B at 4 mg/kg/day led to a higher rate of CSF culture conversion at 2 weeks than conventional amphotericin B.[38] However, a larger, although unpublished, study suggested no significant improvement in sterilization at 2 weeks with liposomal amphotericin B up to 6 mg/kg/day compared with conventional amphotericin B at 0.7 mg/kg/day.[39] Higher doses of liposomal amphotericin B have not been tested in cryptococcal infection, although pharmacokinetic parameters are maximal at 10 mg/kg and doses up to 15 mg/kg have been tolerated in treatment of filamentous fungal infection.[40]

Flucytosine (5-fluorocytosine [5FC]), which was developed as an anticancer drug, was introduced in the 1970s. Within cryptococcal cells, flucytosine is converted by cytosine deaminase (an enzyme present in fungi and bacteria but not in mammalian cells) to 5-fluorouracil (5-FU) a pyrimidine analogue that inhibits nucleic acid synthesis. It is water soluble, well absorbed and penetrates all tissues and the CSF well. In initial studies, monotherapy with flucytosine commonly led to the development of resistance. However, this is not a problem when it is combined with amphotericin B. This combination has also been shown to have additive effects in both pre-clinical studies and clinical trials in non-HIV-associated and HIV-associated infection. In the last large US study, addition of flucytosine 100 mg/kg/day to amphotericin B 0.7 mg/kg/day was associated with a reduced rate of relapse and a trend towards greater sterilization of CSF by 2 weeks.[6] More recently, the fungicidal activity of this combination compared with amphotericin B alone has been directly assessed by means of serial quantitative cultures of the CSF over the first 2 weeks of treatment. The reduction in CSF cryptococcal colony-forming units (CFUs) was found to be exponential, allowing the rate of decrease of CSF CFUs to be calculated from the slope of the linear regression of

log CFU against time for each patient. The mean rate of fall in CFU counts, or early fungicidal activity (EFA), ranged from over half a log reduction per day in CSF CFU for amphotericin B plus flucytosine to less than a third of a log reduction per day in CSF CFU for amphotericin B alone; this difference was highly significant despite the small number of patients studied (Fig. 2).[37] Oral and intravenous flucytosine appeared equally effective and both formulations were well tolerated in this study (A. E. Brouwer and T. S. Harrison, unpublished data).

The main side effects of flucytosine, which are dose dependent, are myelosuppression and gastrointestinal disturbance. In contrast to amphotericin B, flucytosine has concentration-independent activity,[34] prompting testing of lower doses. In two recent trials using a dose of 100 mg/kg/day as opposed to the initial treatment dose of 150 mg/kg/day,

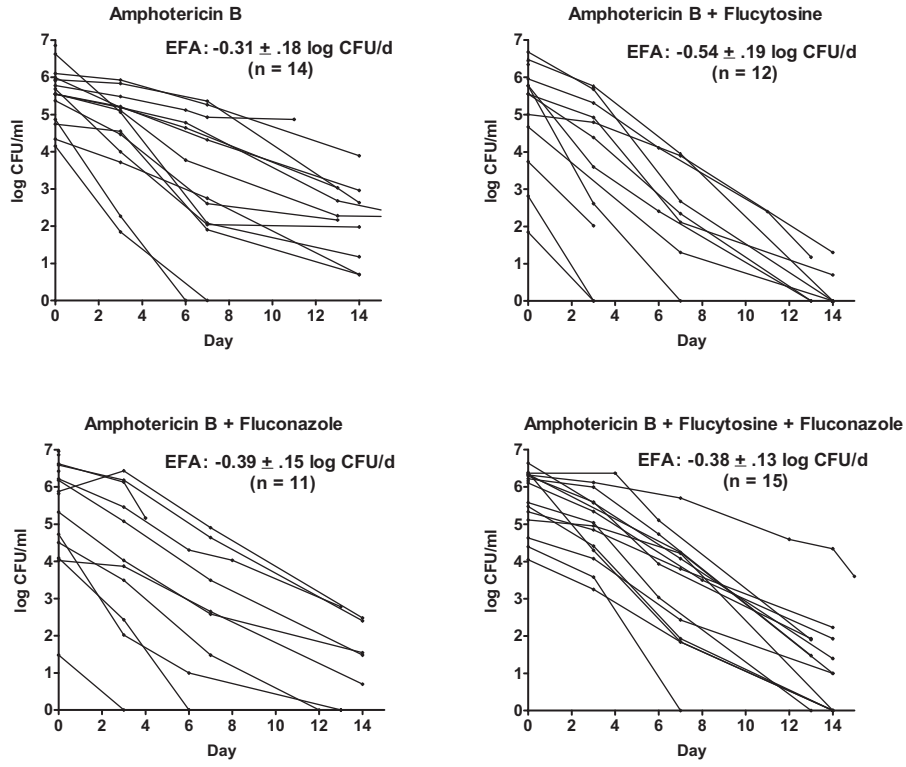


**Fig. 2** Fall in CSF *C.neoformans* CFU over time by treatment group. The decrease in log CFU/ml CSF/day was calculated for each patient using the slope of the linear regression of log CFU against time. For each treatment group, early fungicidal activity (EFA) is shown as the mean ± SD rate of fall in log CFU counts. EFA was significantly greater for AmB plus flucytosine compared with AmB alone ($P < 0.001$), AmB plus fluconazole ($P = 0.02$) or triple therapy with AmB, flucytosine and fluconazole ($P = 0.02$). Reprinted with permission from Elsevier (The Lancet, 2004, Vol 363, pages 1764–1767).

there was no apparent decrease in efficacy and no problem with toxicity, despite monitoring of renal function and haematological parameters only, without drug levels.[6,37] Thus, at this lower dose, flucytosine could be more widely used if it were more readily available. Unfortunately, relatively few pharmaceutical companies have shown an interest in producing the drug.

Fluconazole, a triazole that inhibits fungal ergosterol synthesis, has excellent absorption and CSF penetration and is safe. It is now cheap and widely available in generic form. In a comparative trial using doses lower than those currently recommended, amphotericin B 0.4–0.5 mg/kg/day and fluconazole 200 mg/day had similar outcomes (clinical improvement and negative cultures by 10 weeks, 40% vs 34%; mortality at 10 weeks 14% vs 18%); however, amphotericin B had a shorter median time to CSF sterilization (42 vs 64 days).[41] Low-dose (200 mg/day) fluconazole monotherapy prolonged median survival by only 9 days compared with palliative care alone (19 days vs 10 days) in a Zambian study.[31] The slow response to therapy with fluconazole, essentially a fungistatic drug, prompted the last large Mycoses Study Group trial combining a short 2-week induction phase with amphotericin B (0.7 mg/kg/day), with or without flucytosine (100 mg/kg/day) (see above), with consolidation phase treatment with either fluconazole (400 mg/day) or itraconazole (400 mg/day) for 8 weeks.[6] The idea was to benefit from the more rapid initial action of amphotericin B but then to avoid its toxicity in prolonged administration by switching to an azole. The results were the best to date, with a mortality at 10 weeks of 9.4%, and the trial forms the basis of current treatment guidelines in the USA and Europe (Table 1).[42,43] Itraconazole rather than fluconazole in the consolidation phase was associated with a lower rate of negative cultures at 10 weeks, and in a separate study was found to be less effective than fluconazole as maintenance therapy. Thus fluconazole appears superior in treating cryptococcal disease, although itraconazole remains an alternative oral agent.

**Table 1** Suggested treatment of HIV-associated cryptococcal meningitis

| | Preferred regime[42,43] | Notes |
|---|---|---|
| First 2 weeks | Amphotericin B (AmB) 0.7–1 mg/kg/day + flucytosine 100 mg/kg/day | If flucytosine intolerant or not available, consider AmB 1 mg/kg/day<br>If intolerant of AmB, consider liposomal AmB ≥3 mg/kg/day if available, or switch early to fluconazole |
| Next 8 weeks | Fluconazole 400 mg/day | |
| Thereafter until immune reconstitution (see text) | Fluconazole 200 mg/day | If no facility for i.v. therapy, consider fluconazole 800 mg/day for the first 2 weeks |

Some preclinical and limited clinical data suggest that higher doses of fluconazole may be associated with greater efficacy.[44,45] In small series, median times to sterilization of CSF were 21 days and 33 days for 800 mg/day[46,47] compared with 40 days for 400 mg/day[48] and 64 days for 200 mg/day.[41] Thus, if facilities for administration and monitoring of amphotericin B are not available, fluconazole at 800 mg/day may be preferable to lower doses. Doses of fluconazole up to 2000 mg/day have been tolerated in a small number of patients[45]. Limited data have correlated high fluconazole minimum inhibitory concentration (MIC) (≥16 μg/ml), determined by standardized methods, with failure of fluconazole therapy,[49] and it is important to keep initial isolates so that testing can be undertaken for development of secondary resistance in case of subsequent relapse. To date, primary drug resistance to any of the first-line drugs has not been a major problem, and routine resistance testing may not be a priority in patients who have not had prior azole therapy. Fluconazole is metabolized by and modulates the cytochrome *P*-450 system, and awareness of drug–drug interactions is important. Recent data suggest that fluconazole may increase nevirapine levels, necessitating increased clinical and laboratory vigilance when this combination is used.[50]

While most recent studies, described above, have involved HIV-associated cryptococcal meningitis, a similar induction and consolidation approach is now commonly used in non-HIV-associated infection. In *C.gattii* meningitis in Australia, response to treatment may be slow and amphotericin B, usually with flucytosine, is commonly continued for 4–6 weeks,[51,52] prior to switching to fluconazole. These patients may also require surgical resection of focal pulmonary or CNS lesions and have a higher rate of hydrocephalus requiring shunting. One uncertainty in non-HIV-associated infection is how long to keep patients on fluconazole. In the absence of data, depending on response and whether any immunosuppression can be reversed, most patients are maintained on fluconazole (decreasing to 200 mg/day after 10 weeks total treatment) for 6–12 months.[42] Data on discontinuation of secondary prophylaxis in HIV-infected patients is still limited, but in general fluconazole (200 mg/day) is continued until immune reconstitution with antiretrovirals to a CD4 count >100–200 cells/μl has been sustained for 6–12 months.[53]

### *Raised intracranial pressure*

Significantly raised CSF opening pressure (>25 $cmH_2O$) occurs in >50% of patients with HIV-associated cryptococcal meningitis. This may exist at presentation or develop during mycologically successful therapy, and is associated with worsening headache, altered mental status, visual and

hearing loss, other symptoms and signs of raised intracranial pressure, and increased mortality.[54] A CT or MRI head scan usually shows normal ventricular size and the mechanism is hypothesized to be the obstruction of CSF outflow by a large burden of yeasts and polysaccharide plugging the arachnoid villi.[55] To date, few controlled studies have been completed to evaluate management of this important complication, so that recommendations are based on a few case reports, one small series[56] and expert opinion. After brain imaging to exclude hydrocephalus, marked cerebral oedema or a space-occupying lesion, for patients with an opening pressure of >25 cm, US guidelines suggest daily serial lumbar punctures with withdrawal of large volumes of CSF to achieve a closing pressure of ≤20 cm $H_2O$ or 50% of initial opening pressure.[42] However, even this aggressive approach may be inadequate to control severely elevated opening pressures. Insertion of a temporary lumbar drain, which can safely remove over 200 ml CSF daily in a controlled fashion, can be a much more effective means of pressure control if expertise and facilities allow, and requires further study.[57] Medical treatment using mannitol and acetazolamide has not been shown to be effective. The frequency of cerebral oedema in these patients, and the role, if any, that this may play in the development of raised CSF pressure, is unclear. This limits the rationale for adjunctive corticosteroid use, and, in a retrospective analysis, steroids did not appear to be helpful.[54] In addition, a pro-inflammatory cytokine response in the CSF, which could be compromised by high-dose steroids, is associated with survival and more rapid clearance of infection.[24]

In addition to raised CSF pressure with normal-sized ventricles, patients, especially those with non-HIV-associated cryptococcal meningitis, may present with classical obstructive hydrocephalus or develop this complication during therapy. Neurosurgical shunting improves neurological outcome in this case and, provided that shunting is done after starting antifungal therapy, the prosthetic device has not been found to act as a nidus of infection.[58]

### *Immunotherapy and immune reconstitution*

Patients with cryptococcal meningitis on immunosuppressive medication should have the dose reduced and medication stopped if this is possible. Beyond this, given the frequent underlying defects in immunity in patients with cryptococcal meningitis, there is a strong rationale for adjunctive immunotherapeutic strategies to restore or boost host defence mechanisms.

The precise role of natural antibodies in protection against cryptococcal infection is unclear. Neverthelesss, particular specific monoclonal

antibodies against the cryptococcal capsule polysaccharide have been shown to be beneficial in animal models, and have reached phase I studies in patients recovering from HIV-associated cryptococcal meningitis.[59] Use of radiolabelled antibodies is now being explored in murine models.[60] Adjuvant IFN-γ therapy in AIDS patients with cryptococcal meningitis showed a trend towards improved mycological and clinical success, and was well tolerated with no adverse effects on CD4 count or HIV viral load.[61] Recent studies demonstrating the importance of IFN-γ in the clearance of cryptococci from the CSF[24] support further work on the dosing and scheduling of adjunctive recombinant IFN-γ.

An alternative approach to immunorestoration in patients with HIV-associated infection would be early institution of antiretroviral therapy. On the other hand, such early introduction of antiretrovirals may be associated with an increased risk of immune reconstitution syndromes. Diagnosis of such syndromes is by exclusion and requires the onset of symptoms or signs after initiation of effective antiretroviral treatment, without alternative explanation, attributable to increased reactivity to cryptococcal antigens. Cryptococcal cultures should be negative and histopathology, if available, should show evidence of greater than expected immune reactivity. Manifestations in the setting of cryptococcal infection include fever, lymphadenopathy, new pulmonary infiltrates and recurrent headache with raised CSF pressure despite a negative CSF culture.[62,63] Immune reconstitution syndromes occurred in 8% of a French HIV-infected cohort with cryptococcal meningitis (O. Lortholary *et al*, for the French Cryptococcosis study group, personal communication) at a median of 8 months after diagnosis of cryptococcosis, and were associated with a mortality of 25%. The risk of developing immune reconstitution was highest in those with newly diagnosed HIV infection, a lower CD4 cell count and fungaemia at presentation, and in patients in whom antiretroviral therapy was initiated within 2 months of diagnosis. In view of the potential seriousness of these reactions, in the absence of any controlled studies most experts suggest delaying antiretroviral therapy for at least a month after the diagnosis of cryptococcal meningitis.

## Prognostic factors and outcome

Prior to the HIV epidemic, factors predicting a poor outcome in cryptococcal meningitis included underlying disease (lymphoreticular malignancy or corticosteroid use), no headache, abnormal mental status, high organism burden (Indian ink positivity or cryptococcal antigen titre), poor host inflammatory response (CSF white cell count <20/ml) and raised CSF opening pressure.[64,65] In a more recent series of non-HIV-associated cryptococcal meningitis, after the introduction of fluconazole,

mortality was associated with chronic renal or liver failure or haematologic malignancy as predisposing factors, absence of headache and altered mental status.[17] In *C.gattii* meningitis in Papua New Guinea, death was again associated with altered consciousness, as well as a history of convulsions prior to treatment.[52] In series of HIV-associated cryptococcal meningitis, abnormal mental status and high organism load, measured by quantitative CSF culture or CSF antigen titre, are the most important determinants of death,[37,41] while raised CSF opening pressure and low CSF white cell count are also associated with poor outcome.

The last US Mycoses Study Group treatment trial of HIV-associated cryptococal meningitis had the lowest mortality to date: 9.4% at 10 weeks.[6] However, some of the most severe cases are excluded from such trials and mortality in less selected series has been higher.[7] In Southeast Asia, even in the context of amphotericin-B-based therapy, acute mortality has ranged from 22% to >40%.[37,66] In Africa, outcome data for amphotericin B or high-dose fluconazole are lacking, but results with fluconazole monotherapy at 200 mg/day (median survival 19 days) or fluconazole plus flucytosine in combination (44% mortality at 8 weeks) have been disappointing.[31,67]

In the USA, some studies have suggested that the outcome for non-HIV-associated cryptococcal meningitis may be worse than for HIV-associated infection. Patients with underlying neoplastic disease had shorter survival than AIDS patients even if death due to cryptococcal infection was distinguished from other causes, a result the authors attributed in part to the older age of patients with neoplastic disease.[68] A population-based surveillance study covering the years 1992–2000 found that 21% of patients with non-HIV-associated cryptococccosis died on first admission or within 30 days (in the case of outpatients) compared with 11% of HIV-associated cases.[16]

Despite treatment with amphotericin B plus flucytosine, 34% of patients with *C.gattii* meningitis in Papua New Guinea died during their first admission, at a median of 8 days.[52] In addition, over a third of survivors were left blind and many had hearing loss. A high rate of neurological sequelae has also been documented in series of *C.gattii* infections in Australia.[27,51]

## Prevention

It is probably common sense for high-risk patients to avoid exposure to high concentrations of *C.neoformans* as may be found, for example, in places heavily contaminated with pigeon excreta. Nevertheless, epidemiological studies[18] and the fact that many cases may represent reactivation

of latent infection suggest avoidance of exposure is not a realistic strategy in most cases. Randomized trials and case–control studies have shown that primary antifungal prophylaxis with fluconazole dramatically reduces cryptococcal meningitis in AIDS patients with low CD4 counts.[69,70] Nevertheless such a strategy was never widely adopted in developed countries because of the declining incidence of cryptococcal infection associated with the advent of antiretroviral therapy and concern about development of azole-resistant candidiasis. A study of fluconazole prophylaxis is ongoing in Africa, where the incidence of cryptococcal disease is still very high, and in Thailand many HIV clinics started using fluconazole prophylaxis for patients with a low CD4 count once the generic drug became available and before antiretroviral therapy was widely available. Where and when available, antiretroviral therapy represents the best prophylaxis to prevent HIV-associated cryptococcal disease.

A conjugate cryptococcal glucuronoxylomannan–tetanus toxoid vaccine[71] and capsular polysaccharide peptide mimotope-based vaccines[72] have been shown to be protective in mice, and the former was immunogenic in humans, but its efficacy has not been tested in high-risk patients. Active immunization against *C.neoformans* remains the eventual aim of studies to identify important T-cell epitopes.

## Future work

New azole and echinocandin antifungals have been developed to treat *Candida* and *Aspergillus* infections. Unfortunately, echinocandins have little activity against *C.neoformans* in which the target β1–3 glucan linkage in the cell wall is not important. Voriconazole has good *in vitro* activity against *C.neoformans*, and has been used with good results in some refractory cases of cryptococcosis.[73] However, there are no comparative data yet and animal studies do not suggest a great improvement over fluconazole. Thus immediate prospects for improving antifungal treatment rest largely with exploration of different drug combinations and dosages and working to improve the availability of current drugs in resource-poor areas with the highest burden of disease. The fungicidal activity of novel drug combinations can be accurately assessed with small numbers of patients using serial quantitative CSF cultures to determine the rate of clearance of infection.[37] The most promising regimens could then move forward to clinical endpoint studies. Short courses of higher-dose conventional amphotericin B and high-dose liposomal amphotericin B with or without flucytosine, high-dose fluconazole with or without amphotericin B, amphotericin B plus voriconazole and adjunctive IFN-γ could all be tested in this way. If more rapidly fungicidal

regimes could be defined, the period of induction intravenous therapy could perhaps be reduced to <2 weeks with consequent savings in the cost of hospitalization and intravenous therapy.

Work needs to be done to improve access to antifungal drugs in many areas. While the cost of antiretroviral drugs has reduced markedly in recent years, amphotericin B remains relatively expensive in some countries. For example, in South Africa, 50 mg of amphotericin B costs 140–150 Rand (£12–13), compared with less than £4 in the UK. Flucytosine, although a simple molecule long out of patent, is not readily available in many countries.

Of equal importance to optimizing our use of antifungal drugs, studies are needed to understand the basis of altered mental status in cryptococcal meningitis, given its prognostic significance, and controlled trials are needed to direct the management of raised intracranial pressure.

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