Cryptococcosis

John W. Baddley and William E. Dismukes

Cryptococcosis is a systemic mycosis caused by the encapsulated yeast *Cryptococcus neoformans*, an organism found in soil and often associated with pigeon droppings. Infection involves most frequently the lungs or central nervous system and, less frequently, the blood, skin, skeletal system, and prostate. Because the incidence of cryptococcosis is greatly increased in immunocompromised patients, especially among patients with AIDS or organ transplant recipients, cryptococcosis is considered an opportunistic fungal infection. Treatment of cryptococcosis is based on anatomic site of disease, severity of disease, and the underlying immune status of the patient. Cryptococcal meningitis is treated with induction therapy of amphotericin B with or without flucytosine, followed by a prolonged course of fluconazole. For pulmonary disease alone, fluconazole is effective therapy in most patients. Chronic maintenance therapy with fluconazole may be required in HIV-infected patients or transplant patients who remain immunosuppressed.

Organism

More than 40 species of the genus *Cryptococcus* have been described, but few are recognized as causing infection in humans [1]. The predominant pathogen is *C. neoformans*, but two other species, *C. albidus*, and *C. laurentii*, have been reported to rarely cause disease in humans [2–4]. *Cryptococcus neoformans* is a round or oval encapsulated yeast, measuring approximately 4–6 μm in diameter in clinical specimens, and having a capsule ranging in size from 1 to >30 μm. In specimens isolated from nature, organisms tend to be smaller and poorly encapsulated [5].

Cryptococcus neoformans is grouped into serotypes A, B, C, D, and AD hybrids based on antigenic determinates on the polysaccharide capsule, with serotype A most common. *Cryptococcus neoformans* var. *neoformans* has included serotypes A, D, and AD, and *C. neoformans* var. *gattii* included serotypes B and C. It has been proposed to further simplify the classification of *Cryptococcus* into pathogenic varieties: *C. neoformans* var. *grubii* and *C. neoformans* var. *neoformans. C. neoformans* var. *gattii* has been reclassified as *C. gattii*, a species distinct from *C. neoformans* [6]. The *C. neoformans* varieties differ somewhat in epidemiology, ecology, and certain biochemical properties. In contrast to *C. neoformans*, *C. gattii* uncommonly infects AIDS patients, is found primarily in tropical areas, and is able to assimilate malate. The epidemiology of *C. gattii* is evolving with its emergence in Vancouver, Canada and the Pacific Northwest United States since 1999 [7–9]. For a more detailed description of differences among pathogenic cryptococci , see these two comprehensive references [10, 11].

The sexual, or perfect, state of *C. neoformans*, *Filobasidiella neoformans*, a basidiomycete, can be demonstrated by mating the fungus under certain defined conditions [12]. In this perfect state, mycelia are produced which bear basidiospores 1–3 μm in size. The perfect state has not yet been demonstrated in patients or in nature, so the importance of inhalation of basidiospores in disease acquisition is unknown.

C. neoformans produces white to cream-colored, smooth, mucoid colonies when grown on solid culture media such as blood agar or Sabouraud's dextrose agar. The amount of mucoidness of the colonies is related to the thickness of the capsule. Growth of *Cryptococcus* usually occurs in 36–72 h and is typically slower than that of *Candida* or *Saccharomyces* species under the same conditions. *C. neoformans* grows at 37°C, whereas nonpathogenic species of *Cryptococcus* do not. A distinguishing feature of *C. neoformans* is the ability to produce melanin. On selective media supplemented with niger seed (birdseed agar), smooth brown colonies are formed after several days of incubation. Color reactions on solid media are also useful to distinguish between *C. neoformans* var. *neoformans* or *grubii* and *C. gattii*. For example, colonies of *C. gattii* on canavanine-glycine-bromthymol blue (CGB) agar turn the agar blue, while colonies of *C. neoformans* do not elicit a color change [13].

J.W. Baddley (✉)
Division of Infectious Diseases, Birmingham VA Medical Center, University of Alabama at Birmingham School of Medicine, Birmingham, AL, USA
e-mail: jbaddley@uab.edu

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Epidemiology

C. neoformans is ubiquitous in the environment. The organism was isolated initially in nature from peach juice in 1894 by Francisco Sanefelice, and was first isolated from soil by Emmons in 1951 [14]. *C. neoformans* was isolated from pigeon excrement in 1955, and has since been isolated from multiple geographic sites worldwide, many of which are contaminated by pigeon or other bird excrement. Pigeon droppings are commonly colonized with *C. neoformans*, and may contain greater than 10^6 organisms per gram of fecal material. Pigeons do not appear to develop cryptococcal disease, perhaps due to the pigeon's high body temperature [15]. Although *C. neoformans* is isolated most frequently from pigeon excreta and soil, it has been isolated less commonly from other sources, including fruits and vegetables, decaying wood, dairy products, and excrement from a wide variety of avian species [16].

In contrast to the numerous geographic sites of isolation of *C. neoformans*, the isolation of *C. gattii* has been more restricted [14]. *C. gattii* has been isolated from leaves, wood, bark, and air associated with *Eucalyptus camaldulensis* (red river gum) and few other types of trees, but has not been isolated from bird droppings [16–18]. The distribution of *E. camaldulensis*, in tropical and subtropical regions such as Southern California, Australia, Southeast Asia, Central Africa, and Brazil, corresponds to areas where cases of cryptococcosis due to *C. gattii* are recognized as endemic [14, 19]; however, infections caused by *C. gattii* occur in areas without eucalyptus trees, suggesting an additional unidentified environmental source [20].

Because *C. neoformans* is isolated primarily from pigeon droppings and soil, the assumption has been made that infection arises via aerosolized particles from pigeon excrement. This hypothesis has been difficult to confirm, as most patients who develop cryptococcosis do not recall a history of recent exposure to pigeons or their excreta. Exposure to *C. neoformans*, on the basis of serum antibody levels or skin testing, is common among pigeon handlers; however, the incidence of active cryptococcal infections among this population does not appear to be increased [21, 22]. No particular occupational predisposition to cryptococcosis is currently recognized, although data from population-based surveillance suggest that outdoor occupations may be associated with an increased risk of cryptococcosis [23]. Association with pigeons, pigeon excrement, soil, or dust does not appear to increase the likelihood of proven cryptococcosis [23].

In the majority of cases, infection with *C. neoformans* is thought to be caused by inhalation of the organism, either in yeast form or perhaps as basidiospores, from an environmental source such as bird droppings or soil. Evidence for this mechanism of acquisition is supported by isolation of cryptococci measuring less than 4 μm, ideal for alveolar deposition, from aerosols associated with soil and pigeon excreta [5, 24]. Unlike other mycoses transmitted by aerosolized particles, outbreaks of cryptococcosis from a particular environmental source rarely, if ever, occur [14, 25]. Although lung infection can follow acute inhalation of *Cryptococcus* organisms, in most cases disease occurs as a reactivation of dormant infection.

C. neoformans has been isolated frequently from pulmonary and skin cultures of healthy, asymptomatic individuals, but this fungal organism is not regarded as normal microbial flora in animals or humans [26, 27]. Rarely, skin infection can occur after local inoculation, but in most cases, skin disease results from blood-borne dissemination after an initial lung focus of infection. Person-to-person transmission via inhalation of aerosols has not yet been documented, but in several cases, other sources of presumed human-to-human transmission have been described [28–31]. In one report, a recipient of a corneal transplant from a donor with cryptococcosis developed cryptococcal endophthalmitis more than 2 months after transplantation [28]. In a second case, a healthcare worker developed cryptococcal skin lesions at the site of an inoculation of blood from a patient with cryptococcemia [29]. A more recent case was described in which the recipient of a lung transplant developed cryptococcal left lower lobe pneumonia 2 days after transplantation [30]. Endotracheal cultures from postoperative day 2 were positive for *C. neoformans*, although donor lung cultures were positive only for *Rhodotorula* species; however, development of pulmonary cryptococcosis this early in the post-transplant period suggests transmission by the donor organ. Evidence supporting zoonotic transmission of *Cryptococcus* has been reported [32–34]. In one description, a clinical isolate from a renal transplant recipient with cryptococcal meningitis was indistinguishable on the basis of molecular genotyping from an isolate present in the feces of the patient's pet cockatoo [32].

Cryptococcosis occurs in many patients without a recognized immunologic defect, but the large majority of patients have a predisposing factor or underlying disease [5]. Evidence is convincing that patients with defects in T cell–mediated immunity are at increased risk of developing cryptococcal infection. Predisposing conditions include AIDS, systemic corticosteroids, organ transplantation, lymphoreticular malignancies, diabetes mellitus, pregnancy, and sarcoidosis independent of steroid use (Table 1) [10, 35–39]. With the advent of newer immunosuppressive therapies, especially TNF-α inhibitors and monoclonal antibodies such as alemtuzumab, cryptococcosis is emerging in other patient populations [40–43]. Prior to the AIDS epidemic, up to 50% of patients with cryptococcosis had no recognized T cell immune defect or dysfunction [44, 45]. In a recent observational study of 306 HIV-negative patients with cryptococcosis, 21% had no significant immune dysfunction or other

Table 1 Underlying diseases associated with cryptococcosis

HIV
Corticosteroids
Organ transplantation
Malignancy
CD4 T-cell lymphopenia
Connective tissue disease
Renal failure
Cirrhosis
Chronic lung disease
Immunosuppressive agents (monoclonal antibodies, TNF-α inhibitors)
Diabetes mellitus
Pregnancy
Sarcoidosis
Sytemic lupus erythematosus
Rheumatoid arthritis

predisposing condition to cryptococcosis [35]. In contrast, among patients with a predisposing condition, chronic organ disease and glucocorticosteroid use were most common [35]. Idiopathic CD4 lymphocytopenia has also been associated with cryptococcosis in patients with no other predisposing conditions for cryptococcosis [46, 47].

Before the era of highly active antiretroviral therapy (HAART), the prevalence of cryptococcosis among patients with AIDS was estimated to be between 5% and 10% [48, 49] Data from four United States geographic areas, prior to use of HAART, showed the annual incidence of cryptococcosis among patients with AIDS to range from 17 to 66 cases per 1,000 persons [23]. In contrast, among non-HIV-infected persons, the annual incidence ranged from 0.2 to 0.9 per 100,000 persons. In Europe, the prevalence of cryptococcosis among AIDS patients is lower than that in the United States [50, 51].

Although the widespread use of HAART has lowered the incidence of cryptococcosis cases in medically developed countries, the incidence and mortality rate of cryptococcosis are still extremely high in areas in which there is limited access to HAART and/or healthcare [50–55]. In Africa and other developing areas, the prevalence of cryptococcosis in patients with AIDS approaches 30% and is often an AIDS-defining illness [56]. A recent study estimated that the global burden of HIV-associated cryptococcosis approximates 1 million cases annually worldwide, resulting in more than 600,000 deaths per year by 3 months after infection [57].

Pathogenesis

Once *C. neoformans* is inhaled, transient colonization of the airways occurs before subsequent spread and establishment of respiratory infection. Given the widespread presence of *Cryptococcus* in the environment, exposure is likely common. However, the incidence of infection is very low, suggesting that most people mount an appropriate host response when exposed to the organism. *Cryptococcus*, after it enters the body of a susceptible host, can produce latent infection or acute disease. Development of disease appears to depend on inoculum of inhaled organisms, virulence of the organism, and interaction with the host's cellular immune response. As noted earlier, host defense, especially cell-mediated immunity, is fundamental to protection from cryptococcal infections and is important in containing infection and producing granulomatous inflammation [58].

After inhalation of the organism, the first line of defense is the alveolar macrophage, followed by recruitment of other inflammatory cells via chemokines and cytokines such as IL-12, IL-18, and monocyte chemotactic protein-1 [39]. In addition, complement-mediated phagocytosis appears to have an important role in initial defense [59]. In vitro, alveolar macrophages are able to bind and phagocytize *C. neoformans* in the presence of human serum containing opsonins such as C3 [60]. Macrophages from patients with HIV infection tend to be impaired or defective in both oxidative-dependent and oxidative-independent killing of *C. neoformans* [61].

If initial defense mechanisms in alveoli are ineffective, cryptococci reach the bloodstream and disseminate to other organs, such as the central nervous system (CNS) or prostate. In such sites, additional defense mechanisms are needed to thwart progressive infection. In vitro and in animal models, other cells, including neutrophils, natural-killer cells, macrophage-like microglial cells, and T cell lymphocytes can kill or inhibit growth of cryptococci [62]. Cytokines, especially interleukin-2 and interferon-γ, released by phagocytic cells and lymphocytes, also appear to play an important role in enhancing the killing of *C. neoformans* [10].

The role of humoral immunity in protection against cryptococcal infections is controversial, but increasing data indicate that this facet of the immune response may play an important role. Antibodies to capsular constituents facilitate clearance of cryptococcal antigen, enhancing antibody-dependent cell-mediated killing and increasing antifungal activity of leukocytes and natural killer cells [63, 64]. In addition, an anti-beta-glucan monoclonal antibody has been shown to inhibit growth and capsule formation of *C. neoformans* [65].

Among several factors of virulence and pathogenicity for *C. neoformans* and *C. gattii*, the best characterized include the polysaccharide capsule, thermotolerance (ability to grow at 37°C), melanin pigment production, mannitol production, and soluble extracellular constituents. Several in-depth reviews are recommended for more detailed information about factors of virulence, genetics, and pathogenicity [10, 66–68]. For a particular *C. neoformans* isolate, virulence is attributed to these different factors plus the interaction of the host's immune responses. Three of these factors will be discussed below.

The polysaccharide capsule of *C. neoformans* is composed of a backbone of α-1,3-D-mannopyranose units with single residues of β-D-xylopyranosyl and β-D-glucuronopyranosyl, and referred to as glucuronoxylomannan (GXM). The capsule appears to be the key virulence factor for *C. neoformans*; acapsular mutants are typically avirulent, whereas encapsulated isolates have varying degrees of virulence [69]. The capsule may sometimes protect the organism from host defenses. Phenotypic switching in *Cryptococcus* can occur in vivo during chronic infection, allowing for changes in the polysaccharide capsule and cell wall that affect the yeast's ability to resist phagocytosis [70]. Encapsulated *C. neoformans* cells are not phagocytized or killed by neutrophils, monocytes, or macrophages to the same degree as acapsular mutants [71]. In addition, highly encapsulated strains are less able to stimulate T-cell proliferation, and do not enhance the production of cytokines as well as poorly encapsulated or acapsular strains [72, 73].

Melanin production also appears to be an important virulence factor of *C. neoformans*, based on in vitro and animal in vivo systems. For example, the role of melanin was first demonstrated when naturally occurring *C. neoformans* mutants lacking melanin were found to be less virulent in mice than melanin-producing strains [74]. Melanin is deposited in the inner cell wall of *C. neoformans*, and may resist oxidation or reactive nitrogen intermediates produced by phagocytes [75].

Another virulence mechanism of *Cryptococcus* is its ability to survive within either alkaline or acidic environment of the phagolysosome of phagocytic cells, or bloodstream, thereby allowing it to survive and disseminate. Recent studies suggest glycosphingolipid glucosylceramide is essential for fungal growth in extracellular environments [76, 77].

Clinical Manifestations

Pulmonary Infection

Pulmonary cryptococcal involvement can manifest in a variety of ways, ranging from asymptomatic airway colonization or infection to fulminant respiratory failure with acute respiratory distress syndrome (ARDS) [78, 79]. Most patients are asymptomatic, or will have only mild-to-moderate symptoms such as dyspnea, cough, malaise, pleuritic chest pain, night sweats or, rarely, hemoptysis [80–82]. Constitutional symptoms, such as fever, night sweats, and weight loss are less common in HIV-negative patients unless extrapulmonary disease is also present [80].

In the immunologically normal host, a diagnosis of respiratory colonization with *Cryptococcus* can be made on the basis of a positive respiratory tract culture without evidence of pulmonary symptoms or abnormalities on chest radiography. Limited data suggest that patients with colonization often have underlying pulmonary pathology, such as chronic obstructive pulmonary disease [27]. The diagnosis of colonization, particularly in the immunocompromised patient, must be interpreted with caution. Because of the propensity of *Cryptococcus* for dissemination to the central nervous system (CNS), a thorough evaluation for extrapulmonary sites of cryptococcal involvement in the immunocompromised host is recommended [83].

The radiographic features of pulmonary cryptococcosis are varied and influenced by the degree of immunosuppression of the patient. Findings may reveal lobar, patchy infiltrates (Fig. 1); single or multiple nodular lesions (Fig. 2); interstitial infiltrates; mediastinal or hilar adenopathy

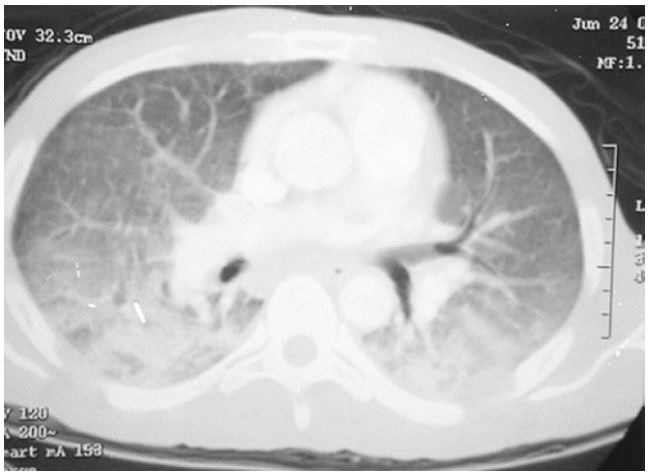

Fig. 1 CT showing severe bilateral cryptococcal lobar pneumonia and prominent adenopathy in an AIDS patient

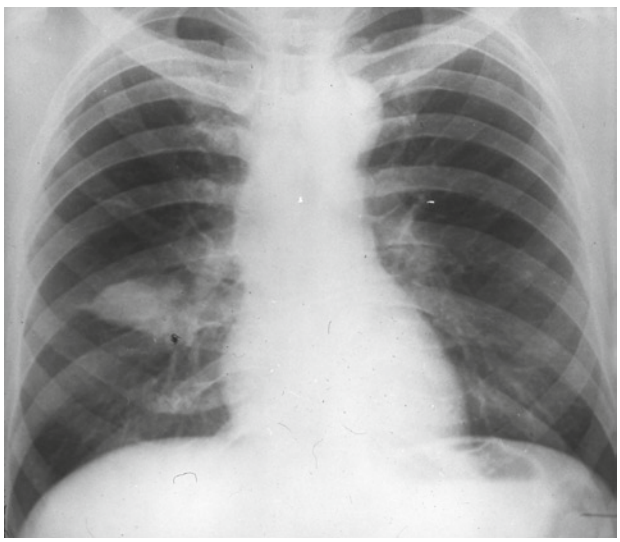

Fig. 2 Well-circumscribed small mass/nodule in patient with underlying systemic lupus erythematosus treated with corticosteroids

(Fig. 3); circumscribed mass lesions (0.5–7 cm) (Fig. 4); or less commonly, pleural effusions or cavitary lesions (Fig. 5) [80, 84]. Radiographically, *C. gattii* infection manifests as focal pulmonary disease and may be mistaken for malignancy [85, 86]. In HIV-negative patients, solitary or multiple pulmonary nodules may be seen in 60–80% of patients [80, 83, 87]. Focal or multifocal airspace consolidation is the next most common radiographic pattern among HIV-negative patients and is present in 10–30% of cases [80, 83, 88]. In contrast, in patients with AIDS, the most common radiographic abnormalities are diffuse, interstitial infiltrates, and lobar, often mass-like, infiltrates [89, 90]. Pulmonary nodules are less common, but are more likely to cavitate than nodules in patients without immune compromise.

Comparison of pulmonary cryptococcal infection in AIDS patients versus HIV-negative patients reveals other important distinctions. In AIDS patients, pulmonary disease plus other sites of involvement are more common. These patients may have a more rapid clinical course, often associated with increased mortality [90, 91]. The majority of AIDS patients with cryptococcal pneumonia have constitutional symptoms, in part explained by increased frequency of concomitant extrapulmonary sites of cryptococcal infection, for example, dissemination to the CNS [90]. The finding of pulmonary

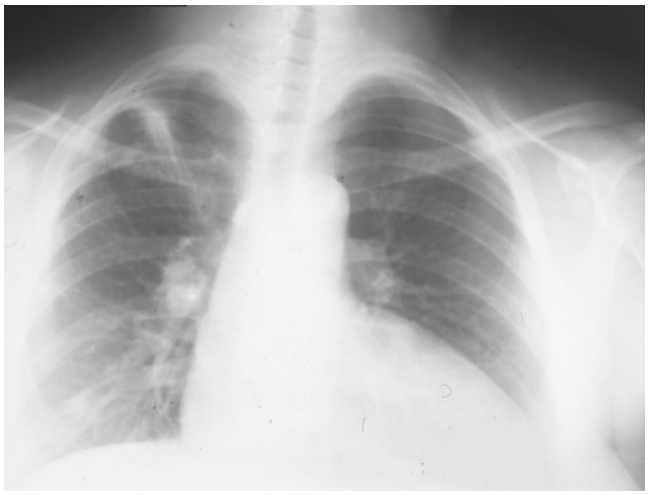

Fig. 3 Cryptococcal lung disease manifest as prominent bilateral hilar adenopathy plus nodule in right upper lobe and patchy pneumonitis in right lower lobe in immunocompetent host

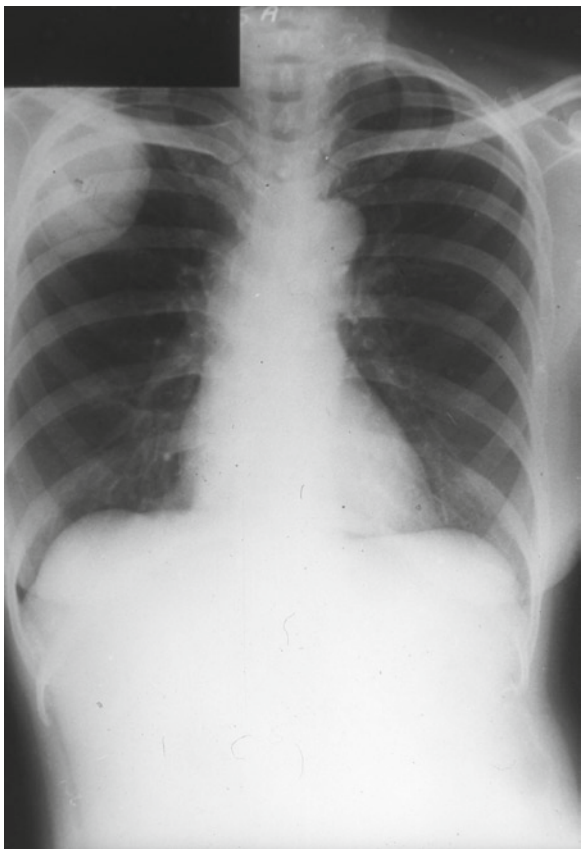

Fig. 4 Large, well-circumscribed cryptococcal mass lesion in right upper lobe of immunocompetent host. Mass was excised surgically

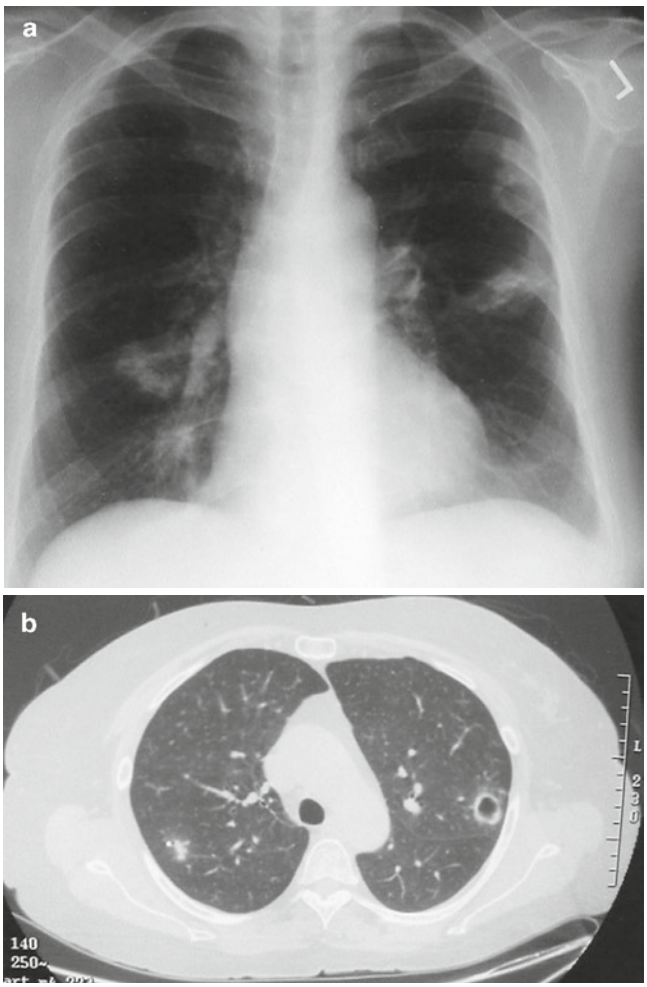

Fig. 5 (**a**) Cryptococcal lung disease manifest as several irregular nodules. (**b**) Note cavitation of left lung nodule shown on CT scan

cryptococcosis in an AIDS patient warrants thorough evaluation for CNS disease, even in the asymptomatic patient.

Central Nervous System Infection

The most common clinical manifestation of cryptococcosis is CNS infection, manifested typically as meningitis, which can be subacute or chronic. The clinical presentation and course of cryptococcal meningitis vary greatly and are often related to the immune status or underlying condition of the patient. In general, the signs and symptoms of cryptococcal meningitis among AIDS patients and HIV-negative patients are similar; however, in AIDS patients, onset tends to be more acute, and the course more rapidly progressive, perhaps explained by poor inflammatory response and the high burden of organisms in these patients. A wide range of symptoms and signs can be seen with CNS cryptococcosis, but complaints are often mild or nonspecific and include headache, nausea and vomiting, and malaise. In some instances, patients are even asymptomatic. Altered mental status, somnolence, and obtundation may signify advanced disease and a poor prognosis [92]. Fever is typically low-grade, and is more likely to be present in HIV-infected patients [92, 93]. Unlike in bacterial meningitis, meningismus is uncommon. Cranial nerve dysfunction may occur in up to 30% of patients, and may result from increased intracranial pressure, cryptococcal invasion of cranial nerves, or brain parenchymal lesions (cryptococcomas). The most common symptoms and signs of cranial nerve involvement include decreased visual acuity, blindness, diplopia, hearing loss, and facial weakness. Seizures, often a reflection of increased intracranial pressure or focal mass lesions, tend to occur later in the course of disease.

Increased intracranial pressure associated with cryptococcal meningitis, especially among patients with AIDS, is a prominent finding. An opening CSF pressure of >250 mm H_2O is found in approximately half of patients with AIDS [94–96]. In AIDS patients, high yeast burden is felt to be a contributing factor to increased intracranial pressures [96]. Cryptococci may cause outflow obstruction by blocking passage of CSF across arachnoid villi. In addition, soluble cryptococcal capsular polysaccharide may accumulate in arachnoid villi, leading to alterations in CSF drainage [94]. Consequently, routine assessment of suspected cryptococcal meningitis should always include manometry. Imaging of the brain is also important to evaluate for hydrocephalus and potential mass lesions; MRI is more effective than CT imaging in identifying lesions [97, 98]. In a recent study evaluating neuroimaging findings in transplant patients with cryptococcosis, outcomes tended to be worse in patients with parenchymal lesions when compared with meningitis or hydrocephalus [99].

Among HIV-negative patients with *C. gattii* infection, intracranial infection is associated with more complications, especially cerebral cryptococcomas, than in patients with *C. neoformans* infection [86, 100]. In AIDS patients, other causes of brain lesions, such as *Toxoplasma gondii* and lymphoma, should be considered. Among AIDS patients with cryptococcal meningitis and increased intracranial pressure, hydrocephalus is an uncommon finding on brain imaging [101]. By contrast, in HIV-negative patients with subacute or chronic cryptococcal meningitis, the course is more likely complicated by hydrocephalus caused by obstruction of flow due to inflammation of the basilar meninges [102]. However, normal ventricular size in the setting of increased intracranial pressure is not uncommon [103].

Prognostic factors for cryptococcal meningitis have been well characterized in both HIV-negative and HIV-infected patients [45, 92, 93, 104, 105]. Important prognostic factors include the patient's underlying disease or predisposing condition, the burden of organisms, titers of CSF cryptococcal antigen, mental status at baseline, and the ability to mount an inflammatory response in CSF [92]. For example, in AIDS patients, fewer white blood cells on initial lumbar puncture may signify poor prognosis [92]. Cryptococcal antigen detection in the CSF may be of prognostic value in certain patient populations. In HIV-negative patients with meningitis, a CSF cryptococcal antigen of ≥ 1:8 at the conclusion of ≥1 month of therapy correlated with likelihood of relapse [45]. Likewise, in AIDS patients, higher titers of CSF cryptococcal antigen (> 1:1024) at baseline are predictive of poorer outcomes [92]. In AIDS patients with meningitis, serial measurement of cryptococcal antigen titers obtained during acute therapy or prolonged suppression has little role in management [106, 107].

Among treated patients with cryptococcal meningitis, mortality rates vary from 5% to 25%, and most deaths occur within the first few weeks of illness [92–94, 108]. Data suggest that mortality in AIDS patients due to cryptococcosis appears to be decreasing, but still remains a common outcome in resource-limited settings [57, 109].

Skin Infection

Cryptococcal skin lesions are seen in up to 15% of patients with disseminated cryptococcosis, and are most common in HIV patients [10]. Skin disease may manifest as a variety of cutaneous lesions, including pustules, papules, purpura, ulcers, cellulitis, superficial granulomas or plaques, abscesses, and sinus tracts [10, 39]. Cases of necrotizing cellulitis have

also been described [110]. In AIDS patients, umbilicated papules resembling molluscum contagiosum are present frequently (Fig. 6) [111]. Cellulitis, characterized by prominent erythema and induration, is often present in patients receiving systemic corticosteroids or other immunosuppressive therapy (Fig. 7) [112]. Cryptococcal skin lesions have resulted rarely from local inoculation, predominately due to laboratory accidents, but the majority of skin lesions result from disseminated infection [113]. Primary cutaneous cryptococcosis appears to be a distinct entity [114].

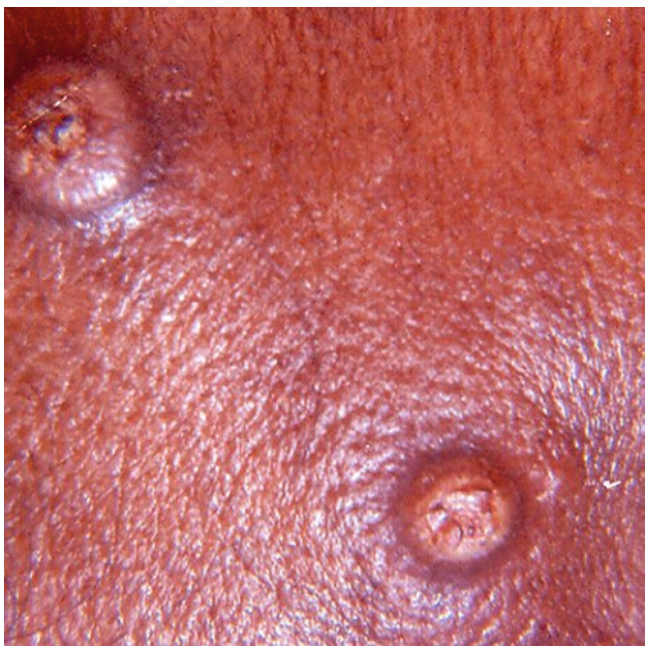

Fig. 6 Molluscum contagiosum–like umbilicated papules due to cryptococcosis in the skin of an AIDS patient

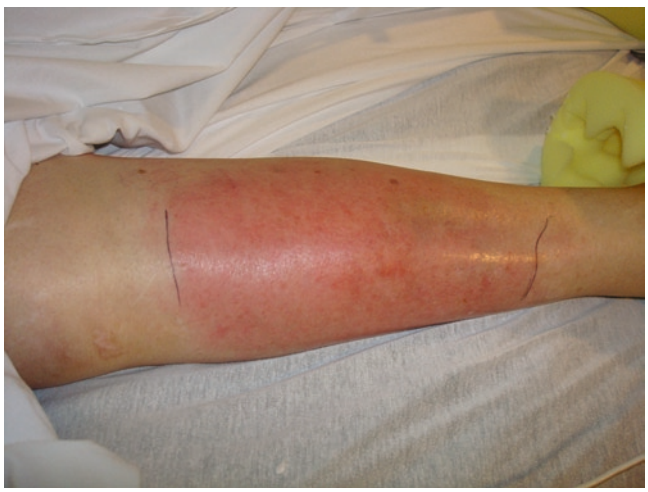

Fig. 7 Cryptococcal cellulitis in a corticosteroid-treated lung transplant patient

Osteoarticular Infection

Cryptococcal lesions of the skeletal system are present in fewer than 10% of patients with disseminated cryptococcosis [115, 116]. Lesions often manifest with soft tissue swelling and tenderness, but lack of symptoms is not uncommon. A single skeletal site is involved most often, with vertebral infection occurring most frequently [115]. On radiography, well-circumscribed, osteolytic lesions, which may resemble malignancy, are seen. Cryptococcal septic arthritis is rare, and most often involves the knee joint [117].

Other Sites of Infection

Cryptococcal infection can involve many other sites and organ systems. Because of the frequency of positive blood cultures and disseminated disease, particularly in AIDS patients, infection may involve virtually any organ. Not infrequently, cryptococcemia in the absence of a proven organ site is discovered [118]. Additional nonmeningeal, extrapulmonary sites of involvement include the prostate, kidneys, muscle, liver, thyroid, sinuses, peritoneum, adrenals, esophagus, heart and aorta, and eyes [39, 119–121]. The prostate gland may also serve as a "sanctuary" for *Cryptococcus* pre- and posttreatment [122, 123]. In one series of HIV-infected patients treated successfully for cryptococcal meningitis, cultures of urine were positive in 9 (22%) of 41 patients at the end of therapy [123].

Immune Reconstitution Inflammatory Syndrome

Immune reconstitution inflammatory syndrome (IRIS) has become an important phenomenon in patients with AIDS and cryptococcal meningitis and recently among transplant recipients [124–127]. IRIS may occur following introduction of HAART in the setting of AIDS with the restoration of CD4 cells or decreasing immunosuppressive therapy in transplant recipients with the reversal of a predominantly Th2 to a Th1 proinflammatory response [128, 129]. Usually, IRIS occurs in one of two scenarios: (1) after starting HAART in patients with cryptococcosis or (2) as a paradoxical effect in patients on HAART during cryptococcal treatment.

The incidence of IRIS is estimated to be between 4 and 16 cases per 100 person-years among AIDS patients [126] and has a prevalence of 4.8% among solid organ transplant recipients [127]. In a recent prospective study evaluating IRIS among 65 HIV patients with cryptococcal meningitis who

started HAART after initiation of antifungal treatment, IRIS developed in 11(17%) patients at a median of 29 days from starting HAART [124]. Patients with IRIS had greater immune responses with HAART (on the basis of T cell recovery) than non-IRIS patients, and IRIS did not appear to be associated with increased mortality rates [124]. Among organ transplant recipients, IRIS has occurred more frequently in patients receiving potent immunosuppressive therapy such as tacrolimus, mycophenolate mofetil, and prednisone when compared with other less immunosuppressive regimens [127].

IRIS may occur within a few days to many months after HAART administration [125]. IRIS typically manifests as meningitis, cryptococcomas, lymphadenitis, or hydrocephalus. Often, symptoms can be confused as signs of treatment failure or disease caused by other opportunistic infections. A positive culture of blood, CSF, or tissue for *C. neoformans*, obtained during evaluation for IRIS, excludes this diagnosis and indicates active cryptococcal infection.

As of yet, there is no reliable way to establish the diagnosis of IRIS; however, risk factors for development of IRIS among AIDS patients include previously unrecognized HIV infection, CD4 cell count <7 cells/μL, fungemia, higher CSF opening pressure, glucose and white blood cell counts, and HAART initiation with 2 months of cryptococcosis diagnosis [125, 126]. Levels of CSF proinflammatory cytokines do not appear to distinguish IRIS patients from those with cryptococcal meningitis alone [124].

Diagnosis

The diagnosis of cryptococcosis can be made by using several methods. A definitive diagnosis is made by culture and identification of the organism from a sterile site. Clinical specimens can be examined with an India ink preparation, a rapid test which is performed by mixing an equal amount of CSF or other fluid and nigrosin or Pelikan India ink on a slide. After adding a coverslip and upon viewing, the polysaccharide capsule of *Cryptococcus* will exclude the ink particles and appear as a halo around the organism (Fig. 8). In patients with cryptococcal meningitis, a positive India ink preparation showing budding yeasts surrounded by a capsule is a useful presumptive test for diagnosis. In AIDS patients with cryptococcal meningitis, India ink preparation of CSF will be positive in 60–80% of cases [92, 93], whereas in HIV-negative patients the positivity rate is lower [35, 130]. Presumptive diagnosis of cryptococcosis can also be made by wet preparations of clinical samples or with the use of Gram stain. However, with these methods, the appearance of cryptococci may be highly variable; therefore, culture should be used for confirmation. Although not specific for the cryptococcal cell wall, Calcofluor white staining may be useful, particularly if few yeast cells are present.

The presumptive diagnosis of cryptococcosis is frequently made on examination of tissue sections. On routine hematoxylin and eosin staining, *C. neoformans* is difficult to identify. However, Gomori-methenamine silver or periodic acid-Schiff staining does allow identification; the organism can be recognized by its oval shape, and narrow-based budding. With the use of mucicarmine staining (Fig. 9), the organism will stain rose to burgundy in color and help differentiate *C. neoformans* from other yeasts, especially *Blastomyces dermatiditis* and *Histoplasma capsulatum.*

The diagnosis of cryptococcal meningitis is easier to establish than the diagnosis of pulmonary cryptococcosis. If cryptococcal meningitis is suspected, a lumbar puncture should be performed. Abnormalities in CSF commonly include elevated opening pressure, hypoglycorrhachia, elevated protein, and a lymphocytic pleocytosis. In AIDS patients with cryptococcal meningitis, the CSF formula

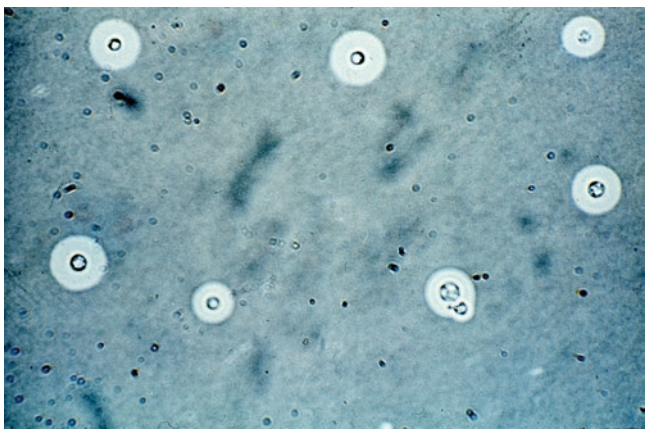

Fig. 8 India ink preparation showing *Cryptococcus neoformans* in cerebrospinal fluid. Note budding yeast form and distinct outline of cell walls and surrounding capsules

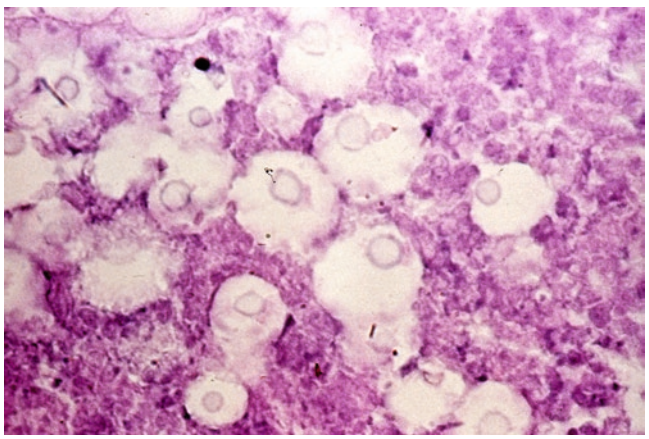

Fig. 9 Mucicarmine stain of brain parenchyma showing numerous densely packed encapsulated cryptococci. Note variable size of capsules

may be normal or show only minimal abnormalities [92]. However, elevated opening pressure is common and may be seen in 50–70% of AIDS patients [94, 96]. In AIDS patients, lack of white blood cells in the CSF is not unusual and may reflect decreased or absent inflammatory response; furthermore, few white blood cells in CSF is a poor prognostic sign [92].

The detection of cryptococcal polysaccharide antigen in CSF or serum is useful in patients with suspected cryptococcosis. After infection is established, cryptococcal polysaccharide becomes solubilized in fluids and can be detected by latex agglutination and quantified. Any positive cryptococcal antigen titer in CSF should be correlated with clinical findings. A titer of ≥ 1:4 strongly suggests cryptococcal infection, particularly in the immunocompromised patient. In cryptococcal meningitis, antigen testing is highly sensitive and specific, and may be particularly useful if CSF cultures are negative. Cryptococcal antigen is found in CSF in >90% and in serum in >70% of patients with cryptococcal meningitis. In AIDS patients with cryptococcal meningitis, sensitivity of the CSF antigen test is even greater (approaching 95–99%) and titers are often higher, up to10^6 [92, 93]. The utility of antigen testing is limited in patients with pulmonary cryptococosis. Among HIV-negative patients with pulmonary cryptococcosis, serum cryptococcal antigen will be positive in 25–56% of cases [35, 131].

With cryptococcal antigen testing, it is important to use proper controls to eliminate errors in testing. The presence of rheumatoid factor may cause a false-positive result when serum is tested, as will the presence of polysaccharide from *Trichosporon asahii* (*beigelii*) or use of inactivated pronase for testing [132]. In addition, false-negative cryptococcal antigen results, although rare, may be due to low numbers of organisms invading the CSF, infection by poorly or non-encapsulated strains, high titers of antigen (prozone phenomenon), low titers of antigen, or immune complexes [10].

Either routine bacteriologic or fungal media will facilitate culture of *C. neoformans*. Colonies are usually detected after 2–7 days of growth. In patients with AIDS and cryptococcal meningitis, blood and CSF cultures will be positive in 55% and 95%, respectively [92, 93]. In a recent study of HIV-negative patients with cryptococcal meningitis, CSF cultures were positive in 89% of patients tested [35]. For blood cultures, the lysis-centrifugation (isolator) technique appears to be the most sensitive method to identify *C. neoformans* [133]. Use of canavanine-glycine-bromthymol blue (CGB) agar will help to distinguish *C. gattii* from other *Cryptococcus* species, as colonies of *C. gattii* will turn the agar blue, while other species do not elicit a color change [13].

The methods of in vitro antifungal susceptibility testing for *C. neoformans* against a variety of antifungal agents have been standardized, although interpretive breakpoints for the antifungal agents against *C. neoformans* have yet to be defined [134]. Most *C. neoformans* isolates appear susceptible to available antifungal agents, including amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole, and posaconazole [135–137]. However, the echinocandin class of antifungals has poor activity against *C. neoformans* [138]. Several reports indicate the potential for microbiologic resistance to fluconazole and clinical failure among patients with cryptococcosis [124, 139–141]. Susceptibility testing should be reserved for patients who have failed primary therapy, for those who relapse after apparently successful primary therapy, and for those who develop cryptococcosis with a history of recent exposure to an azole agent.

Treatment

The treatment of cryptococcosis is decided on the basis of sites of involvement and the underlying immunologic status of the patient. The treatment recommendations and references herein are closely aligned with recent consensus guidelines from the Infectious Diseases Society of America [142]. Please refer to these guidelines for more information. For patients with pulmonary disease, the aims of therapy are to eradicate disease and to prevent dissemination to the CNS. For patients with CNS disease, the aims of therapy are to eradicate or control the infection, adequately manage elevated intracranial pressure, and prevent long-term neurologic sequelae. Prior to 1950, before the availability of amphotericin B, surgical intervention for pulmonary disease was the only therapeutic option available, and cryptococcosis was associated with high mortality rates.

With the availability of amphotericin B in the 1950s and its use as a single agent for cryptococcosis, outcomes were significantly improved, but adverse reactions to amphotericin B were frequently encountered. Flucytosine was found to be an effective drug in vitro for cryptococcosis, and became available for clinical use in 1972 [143]. It was used with moderate success for cryptococcal meningitis and pneumonia; however, single-drug therapy with flucytosine led to rapid emergence of resistance, and flucytosine use as a single agent has, for the most part, been abandoned [143]. Combination therapy with amphotericin B and flucytosine, first employed in a large clinical trial in 1979, resulted in treatment success in 60–85% of patients [45, 130]. In addition, the availability of the triazoles in the early 1990s led to simplification of the primary regimen for cryptococcal meningitis, utilizing, for example, shorter courses of combination amphotericin B and flucytosine followed by prolonged oral therapy with azoles, primarily fluconazole, and good efficacy [92, 93]. Recently, studies of another combination therapy, amphotericin B and fluconazole, have shown encouraging results [144, 145].

Treatment of the HIV-Negative Patient

Pulmonary Infection

The presentation of pulmonary cryptococcosis in the HIV-negative patient can vary widely, ranging from colonization, to asymptomatic disease, to fulminant pneumonia or ARDS [81]. Treatment data from clinical trials among HIV-negative patients with pulmonary infection are limited, and questions remain about which populations require therapy and the optimal dosage and duration of therapy (Table 2) [35, 146, 149]. The only prospective data concerning the treatment of pulmonary cryptococcosis are available from trials involving CNS infections among HIV-negative patients, where resolution of pulmonary disease was not a trial end point [45, 130]. Although few studies, particularly in the era of effective azole therapy, have addressed risk factors for CNS dissemination among patients with pulmonary cryptococcal infection, most authorities recommend lumbar puncture in immunocompromised patients and those with systemic symptoms [81, 83, 142]. A recent study of 166 HIV-negative patients with pulmonary disease identified high-dose corticosteroids, weight loss, headache, and altered mental status as baseline predictors of meningitis [83].

In patients with normal immunologic function and colonization (defined as positive respiratory tract cultures with negative chest radiograph and absence of symptoms), observation is recommended. Immunocompetent patients with an abnormal chest radiograph and asymptomatic disease or mild disease often have done well without therapy, as was common prior to the availability of oral azole antifungals [81, 147, 156]. However, with the availability of oral azole therapy, most immunocompetent patients with pulmonary disease are treated [82].

In contrast, all patients with immune compromise and colonization should receive therapy. If a decision to treat is made, oral fluconazole at a dosage of 400 mg/day for 6–12 months is recommended [146, 148, 149]. In HIV-negative patients with immunocompromising conditions and asymptomatic or mild-to-moderate disease, treatment should be initiated both to prevent CNS dissemination as well as to eradicate symptoms [81, 131]. Fluconazole at a dosage of 400 mg daily is a suitable regimen and is associated with improvement in >80% of cases [35, 146, 149]. Therapy should continue beyond resolution of symptoms and chest radiographic abnormalities. Most experts recommend therapy for 6–12 months' duration [142, 146, 149].

The optimal length of therapy has not yet been determined from clinical trials, but factors to be considered include resolution of symptoms and radiographic findings, persistent immunosuppression, underlying disease, and duration of elevated serum cryptococcal antigen titer. Itraconazole, 200–400 mg daily; voriconazole, 200 mg twice daily; or posaconazole, 400 mg twice daily can be used as alternatives if fluconazole is unavailable or contraindicated [151, 152]. However, there

Table 2 Treatment of cryptococcal Infection in the HIV-negative patient

Pulmonary disease
A. Colonization[a]
1. Observation in the immunocompetent patient
2. Fluconazole 400 mg daily for 6–12 months
B. Asymptomatic or minimally symptomatic disease
1. Fluconazole 200–400 mg/day for 6–12 months [35, 146–148]
Alternative: Close observation without therapy is a consideration
C. Mild-to-moderate disease
1. Fluconazole 200–400 mg/day for 6–12 months [35, 146–149]
Alternative: (1) Itraconazole 200–400 mg/day for 6–12 months [150]
(2) Voriconazole 200 mg twice daily or posaconazole 400 mg twice daily for 6–12 months [151, 152]
D. Severe or progressive disease, or azole drug not an option
Amphotericin B 0.5–1.0 mg/kg/day for a total dose of 1–2 g. This may be followed by oral fluconazole in selected patients [35, 81, 146]
Alternative: (1) Regimens similar to those used for CNS disease, as described below
(2) Surgical resection in selected cases refractory to therapy
CNS disease
Amphotericin B 0.5–1.0 mg/kg/day plus flucytosine 100 mg/kg/day for 2–4 weeks followed by fluconazole 400 mg/day, for 8–10 weeks [93]
Amphotericin B, 0.5–1.0 mg/kg/day plus flucytosine 100 mg/kg/day for 6–10 weeks [45, 130]
Note: Lipid formulations of amphotericin B (liposomal AmB 3–6 mg/kg/day or ABLC 5 mg/kg/day) may be substituted in patients with intolerance to amphotericin B deoxycholate) [153–155]
Adjunctive therapy: see text
Maintenance therapy
Fluconazole 200 mg/day for at least 6 months should be considered for patients with persistent immunosuppression, i.e., transplant recipients [129]

[a]Colonization is defined as a positive respiratory tract culture without signs or symptoms of pulmonary disease or radiographic abnormalities

are few data examining these drugs for the treatment of pulmonary cryptococcosis, much of which evaluates use after treatment failure or drug intolerance.

For HIV-negative patients with severe pulmonary disease or progressive disease, or for whom azole therapy is not an option, most experts would recommend an amphotericin B formulation, with or without flucytosine, as initial therapy for 2–6 weeks, followed by fluconazole [142]. For organ transplant recipients, given the risk of nephrotoxicity associated with concurrent AmB and calcineurin inhibitors, lipid formulations of AmB as induction therapy are preferable for severe non-CNS disease [129].

The role of surgery in patients with pulmonary cryptococcosis is limited [35, 131, 147]. Surgical intervention for pulmonary cryptococcosis may be required for removal of large mass lesions or areas of persistent focal radiographic abnormalities that are refractory to antifungal therapy.

CNS Infection

Early studies among HIV-negative patients with CNS cryptococcosis were important in defining the efficacy of combination therapy with amphotericin B and flucytosine and duration of therapy among immunocompromised patients [45, 130]. In the first prospective study of cryptococcal meningitis in 50 HIV-negative patients, combination therapy with low-dose amphotericin B (0.3 mg/kg/day) plus high-dose flucytosine (150 mg/kg/day) given for 6 weeks was compared to amphotericin B therapy alone (0.4 mg/kg/day) given for 10 weeks [130]. Combination therapy resulted in higher rates of cure and improvement, fewer relapses, and more rapid sterilization of CSF ($P < .001$). Adverse reactions to flucytosine occurred in 11 (32%) of 34 patients, necessitating discontinuation of flucytosine in 6. While the authors concluded that combination therapy was superior to amphotericin B alone, concerns were expressed about the low dosage of amphotericin B and high dosage of flucytosine used in the two arms of the study.

The next prospective study attempted to better address duration of therapy among HIV-negative patients with cryptococcal meningitis by comparing combination therapy with amphotericin B (0.3 mg/kg/day) and flucytosine (150 mg/kg/day) for 4 versus 6 weeks [45]. Note that the treatment regimens employed here were similar to those used in the initial trial [130]. In the second study, 91 patients were randomized to receive either 4 (45 patients) or 6 (46 patients) weeks of therapy. Among randomized patients treated for 4 weeks, cure or improvement was noted in 75%, compared with 85% cure or improvement among patients treated for 6 weeks. Patients who received 4 weeks of therapy had a higher relapse rate (27%) when compared with patients who received 6 weeks of therapy (16%). Toxicities of the regimens in both groups were similar and were most often azotemia, leukopenia, and diarrhea. Among 23 nonrandomized transplant recipients who were protocol-adherent, 16 (70%) of 23 were cured or improved, but 7 (30%) relapsed. From this study, significant baseline predictors of a favorable response included headache, normal mental status, and a CSF white cell count above 20/mm^3. The authors concluded that important considerations in determining duration of therapy should include the patient's underlying disease and immune status and severity of meningitis.

Few other studies are available which address treatment of cryptococcal CNS disease in HIV-negative patients, and none have been randomized or controlled [35, 146]. Dromer and colleagues reviewed retrospectively 83 cases of meningeal and extrameningeal cryptococcosis in HIV-negative French patients, with emphasis on the comparison of efficacy of amphotericin B and fluconazole [146]. Patients with more severe infections, such as meningitis, or those with higher CSF cryptococcal antigen titers, were more likely to receive amphotericin B. However, a subgroup of 25 patients received fluconazole alone for cryptococcal meningitis; 68% were cured with this regimen. A more recent retrospective study by Pappas and colleagues reported findings in 306 patients from 15 US medical centers [35]. As in the Dromer study, patients with CNS disease were more likely to receive amphotericin B, alone or in combination. The most common regimen employed was induction therapy with amphotericin B and flucytosine, followed by consolidation therapy with fluconazole as described for HIV-infected patients. Of 107 patients who received induction therapy with amphotericin B and flucytosine, 90 (84%) were cured or improved. In this study, only 8 of 154 patients with meningitis were treated with fluconazole alone; 7 were cured or improved.

Many of the treatment recommendations for HIV-negative patients with CNS cryptococcal disease have been extrapolated from results of more recent studies in HIV-infected patients [92, 93, 153, 154]. Specific issues addressed by these studies include use of higher doses of amphotericin B (0.5–1.0 mg/kg/day) [92, 93, 155]; substitution of lipid amphotericin B formulations in patients with renal insufficiency [153–155]; and treatment with an "induction" regimen of amphotericin B plus flucytosine for 2 weeks followed by a "consolidation" regimen with fluconazole for an additional 8–12 weeks [93]. For details on these studies, see the section on treatment of CNS disease in HIV-infected patients. For organ transplant recipients, given the risk of nephrotoxicity associated toxicity with concurrent AmB and calcineurin inhibitors, lipid formulations of AmB as induction therapy are preferable for CNS and severe non-CNS disease [129]. The authors favor the "induction/consolidation" approach for the treatment of cryptococcal meningitis in HIV-negative patients, especially for organ transplant recipients [93, 142].

For CNS infections caused by *C. gattii*, treatment recommendations are similar for those patients with CNS infection

secondary to *C. neoformans*. However, because of the frequency of cerebral cryptococcomas in patients with *C. gattii*, management is best guided by imaging studies.

Other Sites of Infection

HIV-negative patients present infrequently with cryptococcal disease at other sites in the absence of pulmonary or CNS infection. Other infections may include skin lesions, abscesses, cryptococcemia, or positive urine cultures. Among solid organ transplant patients, it has been observed that those who received tacrolimus were less likely to have central nervous system involvement and more likely to have skin, soft-tissue, or osteoarticular involvement when compared to patients receiving non tacrolimus-based immunosuppression [157]. In HIV-negative patients, few studies address treatment for these entities [35]. For the majority of patients, treatment is recommended; however, no preferred regimen has been identified. In a retrospective review of 40 HIV-negative patients with cryptococcal disease at non-CNS and nonpulmonary sites, 36 (90%) received antifungal therapy, and 25 (63%) were successfully treated [35]. Multiple regimens were used: 20 evaluable patients received amphotericin B alone or in combination with flucytosine or fluconazole, and 12 (60%) of these 20 were cured or improved; 12 other patients received fluconazole alone and all were cured or improved.

Maintenance Therapy

Although no prospective studies have addressed the use of maintenance or suppressive therapy for HIV-negative patients who have been successfully treated for cryptococcal disease, many experts recommend 6–12 months of additional maintenance therapy with oral fluconazole, 200 mg/day, for selected patients who remain persistently immunocompromised after initial treatment. Among solid organ transplant recipients, the relapse rate of cryptococcosis after 6 months of maintenance therapy is minimal [129]. A recent observation noted that cryptococcal CNS parenchymal lesions may persist radiographically for months or years after completion of therapy, and do not necessarily signify relapse or recurrence of disease [158].

Treatment of the HIV-infected Patient

Pulmonary Infection

The diagnosis of cryptococcal pneumonia in HIV-infected patients is difficult, as the clinical signs and symptoms and radiographic findings can often be nonpecific and mimic disease by other pathogens. Because HIV-related cryptococcal pneumonia is associated frequently with dissemination, a systematic evaluation with blood and CSF cultures and CSF and serum cryptococcal antigen testing is recommended in HIV-infected patients with a positive respiratory tract culture for *Cryptococcus* [142]. Because there have been no controlled trials that evaluate the treatment of pulmonary cryptococcal infection in HIV-infected patients, the treatment of choice and duration of therapy have yet to be elucidated (Table 3).

HIV-infected patients who are asymptomatic, or have mild-to-moderate symptoms with positive respiratory tract cultures, may be good candidates for therapy with oral fluconazole, 200–400 mg daily. Itraconazole, 200–400 mg daily, may be used as a second-line oral therapy [150]. For patients with severe or progressive pulmonary disease, or for patients who cannot tolerate azole therapy, treatment should be similar to recommendations for CNS disease, as described below, and in Table 3.

Length of therapy for cryptococcal pneumonia should be 6–12 months. For HIV-infected patients on HAART with CD4 count >100 cells/μL and decreasing or stable cryptococcal antigen titers (≤ 1:512), discontinuation of maintenance therapy can be considered [166]. For a detailed discussion of this topic, see the section on maintenance therapy under treatment of the HIV-infected patient with CNS infection.

CNS Infection

Many important trials focusing on the treatment of cryptococcal meningitis have been conducted in the HIV-infected population during the last two decades. These studies have demonstrated the efficacy of higher doses of amphotericin B used as primary induction therapy, the safety and efficacy of oral azole antifungal drugs in the treatment of CNS disease, and the importance of adequate management of elevated intracranial pressure associated with cryptococcal meningitis. The principle of rapid fungicidal activity should be the focus of the induction strategy, and a sterile CSF culture at 2 weeks, associated with a favorable outcome, as in previous studies, should be a goal [93, 169].

Based on success rates of 75–85% in earlier studies of combination therapy with amphotericin B and flucytosine among HIV-negative patients with cryptococcal meningitis [45, 130], AIDS patients with CNS disease were treated initially with combination therapy for prolonged periods. However, early reports during the late 1980s suggested that use of flucytosine in HIV-infected patients was frequently associated with cytopenias, and offered no survival benefit or improvement in relapse rate when compared to single therapy with amphotericin B [170]. Because of concerns for flucytosine toxicity, decreased success rates, and the evolving

Table 3 Treatment of cryptococcal infection in the HIV-infected patient

Pulmonary disease
A. Asymptomatic or mild-to-moderate disease
Fluconazole 400 mg/day for 6–12 months depending on immune reconstitution[a] [88, 91, 148]
Alternatives: Itraconazole 400 mg/day 6–12 months depending on immune reconstitution[a] [150]
Fluconazole 400 mg/day plus flucytosine 100 mg/kg/day for 10 weeks [159]
B. Severe, progressive disease
Regimens similar to those for CNS disease (see below)
CNS disease
Amphotericin B 0.7–1.0 mg/kg/day plus flucytosine 100 mg/kg/day for 2 weeks followed by fluconazole 400 mg/day for 8–12 weeks [92, 93]
Alternative: Itraconazole 400 mg/day may be substituted for fluconazole
Amphotericin B 0.7–1.0 mg/kg/day for 4–6 weeks [92]
Amphotericin B 0.7 mg/kg/day plus fluconazole 800 mg/day for ≥8 weeks [145]
Note: Lipid formulations of amphotericin B (liposomal AmB 3–6 mg/kg/day or ABLC 5 mg/kg/day) may be substituted in patients with intolerance to amphotericin B deoxycholate) [153–155]
Fluconazole 400 mg/day plus flucytosine 100 mg/kg/day for 10 weeks [159, 160]
Fluconazole 800–1200 mg/day for 10–12 weeks [161, 162]
Itraconazole 200 mg/day for 10–12 weeks [150, 163]
Maintenance therapy[a]
Fluconazole 200 mg/day [106, 164, 165]
Alternatives: Amphotericin B 1 mg/kg/week [106]
Itraconazole 200–400 mg/day [165]

[a]Lifelong maintenance therapy may be discontinued in selected patients who achieve immune reconstitution with highly active antiretroviral therapy [166–168]

availability of the potent oral azoles fluconazole and itraconazole, subsequent studies evaluated novel regimens for primary therapy of CNS cryptococcal disease [92, 163, 171].

In a small study in the late 1980s of 21 patients with AIDS and cryptococcal meningitis, Larsen and colleagues compared combination therapy with amphotericin B (0.7–1.0 mg/kg/day) plus flucytosine (150 mg/kg/day) to fluconazole (400 mg/day) alone [171]. Clinical and mycologic failure was more common in patients who received fluconazole, particularly in patients with severe disease. In fact, the study was discontinued prematurely because of the higher mortality rate in fluconazole-treated patients. An important finding in this study was the successful treatment in all six patients who received higher doses of amphotericin B as part of the combination regimen. In a second small study of 28 patients with presumed cryptococcal meningitis by De Gans and colleagues, reported in 1992, itraconazole, 200 mg twice daily, was compared to combination therapy with amphotericin B (0.3 mg/kg/day) plus flucytosine (150 mg/kg/day), both administered for 6 weeks [163]. Among patients who received itraconazole, 5 (42%) of 12 achieved a complete response, compared with all 10 patients who received amphotericin B plus flucytosine.

In contrast to these two small trials, two large sequential trials were conducted jointly by the National Institute of Allergy and Infectious Diseases (NIAID) Mycoses Study Group (MSG) and the NIAID AIDS Clinical Trials Group (ACTG) in the 1990s. The initial trial compared amphotericin B (0.3 mg/kg/day) with fluconazole (200 mg/day) in the treatment of AIDS-associated cryptococcal meningitis [92]. Flucytosine as combination therapy with amphotericin B was optional, and was utilized in only nine patients. Treatment was successful in 34% of 131 fluconazole recipients, compared with 40% of 63 amphotericin B recipients. The mortality rate was similar in both groups: 18% in patients who received fluconazole versus 14% in patients who received amphotericin B ($P = .48$). However, the mortality rate during the first 2 weeks was higher among patients receiving fluconazole, and conversion of CSF cultures to negative was less rapid in fluconazole-treated patients. While this study showed no significant difference between the two arms, the results emphasized the need for a more effective primary regimen for the treatment of cryptococcal meningitis.

The second joint study was conducted to evaluate higher doses of amphotericin B, lower doses of flucytosine, and the safety and efficacy of oral azoles in the treatment of AIDS-associated CNS cryptococcosis [93]. Patients were randomized to receive 2 weeks of induction therapy with combination amphotericin B (0.7 mg/kg/day) plus flucytosine (100 mg/kg/day) (202 patients) or amphotericin B alone (0.7 mg/kg/day) (179 patients). At the end of 2 weeks of therapy, if entry criteria were met, patients were again randomized to receive 8 weeks of consolidation treatment with oral fluconazole, 400 mg/day, or oral itraconazole, 400 mg/day. At the end of 2 weeks, CSF cultures for *C. neoformans* were negative in 60% of patients who received combination amphotericin B and flucytosine, compared with 51% of amphotericin B alone treated patients ($P = 0.06$). However, clinical outcomes at 2 weeks did not differ significantly between the two groups. At the end of the 10-week induction and consolidation

treatment period, clinical responses were also similar between the two groups, with 68% of fluconazole-treated patients responding, compared with 70% response among itraconazole-treated patients. Negative CSF cultures were observed in 72% of patients who received fluconazole, compared with 60% of patients who received itraconazole. The addition of flucytosine in the first 2 weeks and treatment with fluconazole over the next 8 weeks were independently associated with CSF sterilization. The use of higher-dose amphotericin B plus lower-dose flucytosine was associated with more effective CSF sterilization and decreased mortality at 2 weeks when compared with previous studies of combination therapy. Fluconazole and itraconazole were both effective as consolidation therapy, although fluconazole appeared to lead to more rapid CSF sterilization. This trial established the concept of "induction" and "consolidation" therapy as an attractive and effective treatment regimen for CNS cryptococcosis.

Additional evidence supporting the efficacy of amphotericin B plus flucytosine as induction therapy treatment was obtained from a randomized trial that evaluated four different antifungal regimens. Sixty-four AIDS patients with first episode cryptococcal meningitis were randomized to receive primary therapy with amphotericin B (0.7 mg/kg daily), amphotericin B (0.7 mg/kg plus flucytosine 100 mg/kg daily), amphotericin B (0.7 mg/kg plus fluconazole 400 mg daily), or a triple-drug regimen consisting of amphotericin B, flucytosine, and fluconazole at the above doses [172]. The primary end point of this study was the rate of reduction in CSF cryptococcal colony-forming units from serial quantitative CSF cultures obtained on days 3, 7, and 14 of treatment. Amphotericin B plus flucytosine achieved clearance of cryptococci from CSF significantly faster than amphotericin B alone, amphotericin B plus fluconazole, and triple-drug therapy. Logistic regression analysis demonstrated that cerebral dysfunction and high counts of *C. neoformans* per milliliter of CSF at baseline were independently associated with early mortality.

A recent cohort study with 208 HIV-positive and -negative patients with cryptococcal meningitis also showed the success of amphotericin B plus flucytosine therapy for 14 days over any other induction regimen among patients with severe cryptococcosis [173]. The risk of failure was 26% in the combination group, compared with 56% with other treatments ($P < 0.001$). Less than 14 days of flucytosine was also independently associated with treatment failure at 3 months in 168 cases of cryptococcosis [173]. A third trial evaluated amphotericin B (0.7 mg/kg/day vs 1.0 mg/kg/day) combined with flucytosine; the regimen utilizing higher-dose amphotericin B was more fungicidal with manageable toxicity, but there was no difference in 2- and 10-week mortality [108].

For HIV-infected patients with cryptococcal meningitis and renal insufficiency, lipid formulations of amphotericin B may be substituted. Clinical experience suggests that combination therapy with lipid formulations of amphotericin B is effective; however, only a few trials have evaluated these formulations [153–155, 174]. The optimal dosages of lipid formulations of amphotericin B have not yet been determined. Response rates of 66–86% were seen in patients receiving amphotericin B lipid complex [153, 174], and in 80% of patients receiving liposomal amphotericin B at a dose of 4 mg/kg/day [155]. An additional study with liposomal amphotericin B showed clinical response in 18 (78%) of 23 patients [154].

Although less well studied, other therapeutic options for CNS cryptococcal disease in HIV-infected patients have been employed, but are considered second-line options to the induction/consolidation options above. In a very recent study of 143 randomized patients, the combination use of amphotericin B (0.7 mg/kg/day) plus fluconazole (800 mg/day) demonstrated satisfactory outcomes compared to amphotericin B alone and may be a reasonable approach to therapy in settings where flucytosine is not available or contraindicated [145]. In this phase II study, the 14-day end point of success in the amphotericin B alone, amphotericin B plus fluconazole 400 mg/day (6 mg/kg), and amphotericin B plus fluconazole 800 mg/day (12 mg/kg) was 41%, 27%, and 54%, respectively. If this combination is used, the higher fluconazole dose is recommended. Combination therapy with fluconazole and flucytosine appears more effective than fluconazole alone, but is also more toxic [159, 160]. Primary therapy with either fluconazole or itraconazole alone administered for 10–12 weeks has also been evaluated in several trials, with variable responses [92, 150, 161–163]. If fluconazole is used alone, then higher daily doses should be administered. Notably, low success rates at 800 mg/day have been substantially improved with 1,200–2,000 mg/day [175]. When using higher daily doses of fluconazole, divided doses are recommended to minimize gastrointestinal toxicity.

Maintenance Therapy

After initial successful treatment of cryptococcal meningitis in AIDS patients, high relapse rates have been demonstrated in patients who did not receive lifelong suppressive or chronic maintenance therapy [164]. A placebo-controlled trial evaluated the effectiveness of fluconazole as maintenance therapy for AIDS patients who received successful therapy for cryptococccal meningitis with amphotericin B with or without flucytosine [164]. Relapse occurred in 15% of patients in the placebo group, compared with 0% in fluconazole-treated patients, thereby establishing the need for maintenance therapy in this population.

Subsequently, a randomized comparative trial conducted by the NIAID-MSG and NIAID-ACTG demonstrated the

superior efficacy of oral fluconazole (200 mg daily) to intravenous amphotericin B (1 mg/kg weekly) for maintenance therapy [106]. Relapses of symptomatic cryptococcal disease were seen in 18% and 2% of patients receiving amphotericin B and fluconazole, respectively ($P < .001$). In addition, patients receiving amphotericin B had more frequent adverse events and associated bacterial infections.

The NIAID-MSG and NIAID-ACTG conducted another trial comparing oral fluconazole (200 mg daily) with oral itraconazole (200 mg daily) for 12 months as maintenance therapy for CNS cryptococcal disease [165]. Fluconazole proved to be superior; the trial was terminated prematurely after interim analysis revealed that 23% of itraconazole-treated patients relapsed, compared with only 4% of fluconazole-treated patients ($P = .006$). Furthermore, the trial showed that risk of relapse was increased if the patient had not received flucytosine during the initial 2 weeks of primary therapy for cryptococcal meningitis ($P = .04$). These studies established fluconazole as the drug of choice for maintenance of cryptococcal disease in HIV-infected patients.

Until recently, maintenance or lifelong suppressive therapy has been recommended for all AIDS patients after successful completion of therapy for acute cryptococcosis [166]. Studies suggest that risk of recurrence of cryptococcosis in AIDS patients is low, provided patients have successfully completed primary therapy for cryptococcosis, are free of symptoms of cryptococcosis, and have achieved immune reconstitution with HAART therapy [167, 168, 176]. For example, a prospective, multicenter trial conducted among 42 AIDS patients in Thailand randomized patients to continue or discontinue maintenance fluconazole therapy when the CD4 cell count had increased to >100 cells/μL and an undetectable HIV RNA level had been sustained for 3 months [168]. At a median of 48 weeks of observation, there were no episodes of relapse of cryptococcal meningitis in either group.

A second retrospective multicenter study was conducted among 100 patients with AIDS living in six different countries [167]. Inclusion criteria were a proven diagnosis of cryptococcal meningoencephalitis, a CD4 cell count of >100 cells/μL while receiving HAART, and the subsequent discontinuation of maintenance antifungal therapy. No relapse or death occurred during a median period of 26.1 months when patients were receiving both HAART and maintenance therapy for cryptococcal meningitis. After discontinuation of maintenance therapy, four relapses occurred (incidence 1.53 cases per 100 person-years) during a median period of observation of 28.4 months. This illustrates that careful follow-up of patients who discontinue maintenance therapy is necessary.

Collectively, these results indicate that maintenance therapy for cryptococcal meningitis may be safely discontinued in most patients (1) responding to HAART with a CD4 cell count >100/μL and an undetectable or low HIV RNA level sustained for ≥3 months and (2) receiving at least 1 year of antifungal drug exposure with close patient follow-ups and serial cryptococcal serum antigen tests. Reinstitution of fluconazole maintenance therapy should be considered if CD4 count drops below 100/μL and/or serum cryptococcal antigen titer rise. Some authorities recommend a lumbar puncture to confirm CSF sterility prior to discontinuation of maintenance therapy.

The decision of when to initiate HAART in the treatment of coinfection with *Cryptococcu*s and HIV to avoid IRIS remains uncertain. Recent studies suggest earlier initiation of HAART within 2 weeks may be possible without triggering an unacceptable increase in the frequency or severity of IRIS [177]. In some clinical settings, long delays in HAART can place patients at risk for dying of other complications of HIV infection. It is also important to anticipate complications of drug interactions with HAART and antifungal drugs.

Adjunctive Therapy

As mentioned previously, elevated intracranial pressure is a common finding in cryptococcal meningoencephalitis, especially among patients with AIDS. Furthermore, a very high opening pressure at baseline may be associated with more frequent headaches and meningismus, pathologic reflexes, early death, and overall increased mortality rates [94]. The treatment of persistent elevated intracranial pressure is aimed at reducing CSF volume, either by repeated lumbar puncture or lumbar or ventricular drainage. If intracranial pressures cannot be adequately reduced with frequent lumbar punctures, lumbar drain placement or ventriculostomy may be necessary for CSF removal [102]. Intraventricular shunting, via ventriculoperitoneal shunt, is often reserved for patients with hydrocephalus or persistently elevated pressures. In HIV-negative patients with persistently increased intracranial pressure and no evidence of hydrocephalus on imaging studies, placement of a ventriculoperitoneal shunt may also be life-saving [103]. Moreover, shunting is not typically associated with dissemination of cryptococcal infection into the peritoneum or bloodstream [102]. Medical therapy including the use of acetazolamide, mannitol, or corticosteroids is not useful in the management of increased intracranial pressure in cryptococcal meningoencephalitis [178].

Prevention and Control

Prevention of cryptococcal disease is difficult because *C. neoformans* is ubiquitous in the environment and only causes sporadic disease. Because of the morbidity and mortality

associated with CNS cryptococcal disease, and the increased incidence of cryptococcosis in patients with advanced HIV infection, primary prophylaxis has been studied in AIDS patients in several prospective trials [179, 180]. Although fluconazole and itraconazole have been shown to reduce the frequency of primary cryptococcal disease among those who have CD4 counts <50 cells/μL, a survival benefit has not been established. In addition, concern exists for the development of azole-resistant fungi if widespread antifungal prophylaxis is employed. For these reasons and the concern for drug interactions, medication compliance, and costs, primary prophylaxis for crytococcosis is not routinely recommended [179, 180]. However, in areas in which availability of HAART is limited, HIV drug resistance is high, and incidence of cryptococcal disease is very high, prophylaxis or pre-emptive strategies with use of serum cryptococcal antigen might be considered [181].

Asymptomatic cryptococcal antigenemia is a well-documented clinical condition in advanced HIV disease, and its prevalence has ranged between 4% and 12% per year in certain populations [182–186]. Antigenemia preceded symptoms of meningitis by a median of 22 days in one study in Uganda and, when not detected, made appearance of disease unlikely over the subsequent year [187]. Cryptococcal antigenemia has been shown to be associated with increased mortality rates among those initiating HAART, and persons with antigenemia are at theoretical risk for the "unmasking" form of cryptococcal IRIS [128]. The precise management of asymptomatic antigenemia remains uncertain, but an aggressive diagnostic and pre-emptive therapeutic stance may be warranted in areas with increased incidence.

References

1. de Hoog G. Atlas of Clinical Fungi. 2nd ed. Washington, DC: American Society for Microbiology Press; 2001.
2. Johnson LB, Bradley SF, Kauffman CA. Fungaemia due to *Cryptococcus laurentii* and a review of non-neoformans cryptococcaemia. Mycoses. 1998;41:277–80.
3. Khawcharoenporn T, Apisarnthanarak A, Mundy LM. Non-neoformans cryptococcal infections: a systematic review. Infection. 2007;35:51–8.
4. Kordossis T, Avlami A, Velegraki A, et al. First report of *Cryptococcus laurentii* meningitis and a fatal case of *Cryptococcus albidus* cryptococcaemia in AIDS patients. Med Mycol. 1998;36:335–9.
5. Neilson JB, Fromtling RA, Bulmer GS. *Cryptococcus neoformans*: size range of infectious particles from aerosolized soil. Infect Immun. 1977;17:634–8.
6. Franzot SP, Salkin IF, Casadevall A. *Cryptococcus neoformans* var. *grubii:* separate varietal status for *Cryptococcus neoformans* serotype A isolates. J Clin Microbiol. 1999;37:838–40.
7. Kidd SE, Hagen F, Tscharke RL, et al. A rare genotype of *Cryptococcus gattii* caused the cryptococcosis outbreak on Vancouver Island (British Columbia, Canada). Proc Natl Acad Sci USA. 2004;101:17258–63.
8. Chambers C, MacDougall L, Li M, Galanis E. Tourism and specific risk areas for *Cryptococcus gattii*, Vancouver Island, Canada. Emerg Infect Dis. 2008;14:1781–3.
9. Byrnes 3rd EJ, Bildfell RJ, Frank SA, Mitchell TG, Marr KA, Heitman J. Molecular evidence that the range of the Vancouver Island outbreak of *Cryptococcus gattii* infection has expanded into the Pacific Northwest in the United States. J Infect Dis. 2009; 199:1081–6.
10. Mitchell TG, Perfect JR. Cryptococcosis in the era of AIDS–100 years after the discovery of *Cryptococcus neoformans*. Clin Microbiol Rev. 1995;8:515–48.
11. Kwon-Chung KJ, Bennett JE. Epidemiologic differences between the two varieties of *Cryptococcus neoformans*. Am J Epidemiol. 1984;120:123–30.
12. Kwon-Chung KJ. A new species of Filobasidiella, the sexual state of *Cryptococcus neoformans* B and C serotypes. Mycologia. 1976; 68:943–6.
13. Min KH, Kwon-Chung KJ. The biochemical basis for the distinction between the two *Cryptococcus neoformans* varieties with CGB medium. Zbl Bakt Mik Hyg. 1986;261:471–80.
14. Levitz SM. The ecology of *Cryptococcus neoformans* and the epidemiology of cryptococcosis. Rev Infect Dis. 1991;13:1163–9.
15. Emmons CW. Saprophytic sources of *Cryptococcus neoformans* associated with the pigeon (*Columba livia*). Am J Hyg. 1955;62: 227–32.
16. Girish Kumar KCP, Prabu D, Mitani H, Mikami Y, Menon T. Environmental isolation of *Cryptococcus neoformans* and *Cryptococcus gattii* from living trees in Guindy National Park, Chennai, South India. Mycoses. 2010;53:262–4.
17. Refojo N, Perrotta D, Brudny M, Abrantes R, Hevia AI, Davel G. Isolation of *Cryptococcus neoformans* and *Cryptococcus gattii* from trunk hollows of living trees in Buenos Aires City, Argentina. Med Mycol. 2009;47:177–84.
18. Granados DP, Castaneda E. Isolation and characterization of *Cryptococcus neoformans* varieties recovered from natural sources in Bogota, Colombia, and study of ecological conditions in the area. Microb Ecol. 2005;49:282–90.
19. Ellis DH, Pfeiffer TJ. Natural habitat of *Cryptococcus neoformans* var. *gattii*. J Clin Microbiol. 1990;28:1642–4.
20. Chen SC, Currie BJ, Campbell HM, et al. *Cryptococcus neoformans* var. *gatti*i infection in northern Australia: existence of an environmental source other than known host eucalypts. Trans R Soc Trop Med Hyg. 1997;91:547–50.
21. Fink JN, Barboriak JJ, Kaufman L. Cryptococcal antibodies in pigeon breeders' disease. J Allergy. 1968;41:297–301.
22. Newberry Jr WM, Walter JE, Chandler Jr JW, Tosh FE. Epidemiologic study of *Cryptococcus neoformans*. Ann Intern Med. 1967;67:724–32.
23. Hajjeh RA, Conn LA, Stephens DS, Cryptococcal Active Surveillance Group, et al. Cryptococcosis: population-based multistate active surveillance and risk factors in human immunodeficiency virus-infected persons. J Infect Dis. 1999;179:449–54.
24. Powell KE, Dahl BA, Weeks RJ, Tosh FE. Airborne *Cryptococcus neoformans*: particles from pigeon excreta compatible with alveolar deposition. J Infect Dis. 1972;125:412–5.
25. Swinne D, Deppner M, Laroche R, Floch JJ, Kadende P. Isolation of *Cryptococcus neoformans* from houses of AIDS-associated cryptococcosis patients in Bujumbura (Burundi). AIDS (London). 1989;3:389–90.
26. Duperval R, Hermans PE, Brewer NS, Roberts GD. Cryptococcosis, with emphasis on the significance of isolation of *Cryptococcus neoformans* from the respiratory tract. Chest. 1977;72:13–9.
27. Tynes B, Mason KN, Jennings AE, Bennett JE. Variant forms of pulmonary cryptococcosis. Ann Intern Med. 1968;69:1117–25.
28. Beyt Jr BE, Waltman SR. Cryptococcal endophthalmitis after corneal transplantation. N Engl J Med. 1978;298:825–6.

29. Glaser JB, Garden A. Inoculation of cryptococcosis without transmission of the acquired immunodeficiency syndrome. N Engl J Med. 1985;313:266.
30. Kanj SS, Welty-Wolf K, Madden J, et al. Fungal infections in lung and heart-lung transplant recipients. Report of 9 cases and review of the literature. Medicine. 1996;75:142–56.
31. de Castro LE, Sarraf OA, Lally JM, Sandoval HP, Solomon KD, Vroman DT. *Cryptococcus albidus* keratitis after corneal transplantation. Cornea. 2005;24:882–3.
32. Nosanchuk JD, Shoham S, Fries BC, Shapiro DS, Levitz SM, Casadevall A. Evidence of zoonotic transmission of *Cryptococcus neoformans* from a pet cockatoo to an immunocompromised patient. Ann Intern Med. 2000;132:205–8.
33. Lagrou K, Van Eldere J, Keuleers S, et al. Zoonotic transmission of *Cryptococcus neoformans* from a magpie to an immunocompetent patient. J Intern Med. 2005;257:385–8.
34. Shrestha RK, Stoller JK, Honari G, Procop GW, Gordon SM. Pneumonia due to *Cryptococcus neoformans* in a patient receiving infliximab: possible zoonotic transmission from a pet cockatiel. Respir Care. 2004;49:606–8.
35. Pappas PG, Perfect JR, Cloud GA, et al. Cryptococcosis in human immunodeficiency virus-negative patients in the era of effective azole therapy. Clin Infect Dis. 2001;33:690–9.
36. Vilchez RA, Irish W, Lacomis J, Costello P, Fung J, Kusne S. The clinical epidemiology of pulmonary cryptococcosis in non-AIDS patients at a tertiary care medical center. Medicine. 2001;80:308–12.
37. Sun HY, Wagener MM, Singh N. Cryptococcosis in solid-organ, hematopoietic stem cell, and tissue transplant recipients: evidence-based evolving trends. Clin Infect Dis. 2009;48:1566–76.
38. Kontoyiannis DP, Peitsch WK, Reddy BT, et al. Cryptococcosis in patients with cancer. Clin Infect Dis. 2001;32:e145–50.
39. Chayakulkeeree M, Perfect JR. Cryptococcosis. Infect Dis Clin N Am. 2006;20:507–44.
40. Hage CA, Wood KL, Winer-Muram HT, Wilson SJ, Sarosi G, Knox KS. Pulmonary cryptococcosis after initiation of anti-tumor necrosis factor-alpha therapy. Chest. 2003;124:2395–7.
41. Silveira FP, Husain S, Kwak EJ, et al. Cryptococcosis in liver and kidney transplant recipients receiving anti-thymocyte globulin or alemtuzumab. Transpl Infect Dis. 2007;9:22–7.
42. Peleg AY, Husain S, Kwak EJ, et al. Opportunistic infections in 547 organ transplant recipients receiving alemtuzumab, a humanized monoclonal CD-52 antibody. Clin Infect Dis. 2007;44:204–12.
43. Munoz P, Giannella M, Valerio M, et al. Cryptococcal meningitis in a patient treated with infliximab. Diagn Microbiol Infect Dis. 2007;57:443–6.
44. Lewis JL, Rabinovich S. The wide spectrum of cryptococcal infections. Am J Med. 1972;53:315–22.
45. Dismukes WE, Cloud G, Gallis HA, et al. Treatment of cryptococcal meningitis with combination amphotericin B and flucytosine for four as compared with six weeks. N Engl J Med. 1987;317:334–41.
46. Zonios DI, Falloon J, Huang CY, Chaitt D, Bennett JE. Cryptococcosis and idiopathic CD4 lymphocytopenia. Medicine (Baltimore). 2007;86:78–92.
47. Duncan RA, von Reyn CF, Alliegro GM, Toossi Z, Sugar AM, Levitz SM. Idiopathic CD4+ T-lymphocytopenia – four patients with opportunistic infections and no evidence of HIV infection. N Engl J Med. 1993;328:393–8.
48. Currie BP, Casadevall A. Estimation of the prevalence of cryptococcal infection among patients infected with the human immunodeficiency virus in New York City. Clin Infect Dis. 1994;19:1029–33.
49. Dismukes WE. Antifungal therapy: from amphotericin B to present. Trans Am Clin Climatol Assoc. 1993;104:166–77. discussion 77–9.
50. Dromer F, Mathoulin S, Dupont B, Laporte A, French Cryptococcosis Study Group. Epidemiology of cryptococcosis in France: a 9-year survey (1985–1993). Clin Infect Dis. 1996;23:82–90.
51. Dromer F, Mathoulin-Pelissier S, Fontanet A, Ronin O, Dupont B, Lortholary O. Epidemiology of HIV-associated cryptococcosis in France (1985–2001): comparison of the pre- and post-HAART eras. AIDS (London). 2004;18:555–62.
52. Mirza SA, Phelan M, Rimland D, et al. The changing epidemiology of cryptococcosis: an update from population-based active surveillance in 2 large metropolitan areas, 1992–2000. Clin Infect Dis. 2003;36:789–94.
53. Chen SC. Cryptococcosis in Australasia and the treatment of cryptococcal and other fungal infections with liposomal amphotericin B. J Antimicrob Chemother. 2002;49 Suppl 1:57–61.
54. Hakim JG, Gangaidzo IT, Heyderman RS, et al. Impact of HIV infection on meningitis in Harare, Zimbabwe: a prospective study of 406 predominantly adult patients. AIDS (London). 2000;14:1401–7.
55. Jarvis JN, Boulle A, Loyse A, et al. High ongoing burden of cryptococcal disease in Africa despite antiretroviral roll out. AIDS (London). 2009;23:1182–3.
56. Heyderman RS, Gangaidzo IT, Hakim JG, et al. Cryptococcal meningitis in human immunodeficiency virus-infected patients in Harare, Zimbabwe. Clin Infect Dis. 1998;26:284–9.
57. Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS (London). 2009;23:525–30.
58. Goldman D, Lee SC, Casadevall A. Pathogenesis of pulmonary *Cryptococcus neoformans* infection in the rat. Infect Immun. 1994;62:4755–61.
59. Kwon-Chung KJ, Kozel TR, Edman JC, et al. Recent advances in biology and immunology of *Cryptococcus neoformans*. J Med Vet Mycol. 1992;30 Suppl 1:133–42.
60. Bolanos B, Mitchell TG. Phagocytosis of *Cryptococcus neoformans* by rat alveolar macrophages. J Med Vet Mycol. 1989;27:203–17.
61. Harrison TS, Levitz SM. Mechanisms of impaired anticryptococcal activity of monocytes from donors infected with human immunodeficiency virus. J Infect Dis. 1997;176:537–40.
62. Levitz SM, Mathews HL, Murphy JW. Direct antimicrobial activity of T cells. Immunol Today. 1995;16:387–91.
63. Casadevall A. Antibody immunity and invasive fungal infections. Infect Immun. 1995;63:4211–8.
64. Lendvai N, Casadevall A, Liang Z, Goldman DL, Mukherjee J, Zuckier L. Effect of immune mechanisms on the pharmacokinetics and organ distribution of cryptococcal polysaccharide. J Infect Dis. 1998;177:1647–59.
65. Rachini A, Pietrella D, Lupo P, et al. An anti-beta-glucan monoclonal antibody inhibits growth and capsule formation of *Cryptococcus neoformans* in vitro and exerts therapeutic, anticryptococcal activity in vivo. Infect Immun. 2007;75:5085–94.
66. Buchanan KL, Murphy JW. What makes *Cryptococcus neoformans* a pathogen? Emerg Infect Dis. 1998;4:71–83.
67. Ma H, May RC. Virulence in *Cryptococcus* species. Adv Appl Microbiol. 2009;67:131–90.
68. Heitman J, Kozel T, Kwon-Chung J, Perfect J, Casadevall A, editors. *Cryptococcus*: From human pathogen to model yeast. Washington, DC: American Society for Microbiology Press; 2010.
69. Chang YC, Kwon-Chung KJ. Complementation of a capsule-deficient mutation of *Cryptococcus neoformans* restores its virulence. Mol Cell Biol. 1994;14:4912–9.
70. Guerrero A, Jain N, Goldman DL, Fries BC. Phenotypic switching in *Cryptococcus neoformans*. Microbiology. 2006;152:3–9.
71. Bulmer GS, Sans MD. *Cryptococcus neoformans*. 3. Inhibition of phagocytosis. J Bacteriol. 1968;95:5–8.
72. Levitz SM, Tabuni A, Kornfeld H, Reardon CC, Golenbock DT. Production of tumor necrosis factor alpha in human leukocytes stimulated by *Cryptococcus neoformans*. Infect Immun. 1994;62:1975–81.

73. Collins HL, Bancroft GJ. Encapsulation of *Cryptococcus neoformans* impairs antigen-specific T-cell responses. Infect Immun. 1991;59:3883–8.
74. Kwon-Chung KJ, Polacheck I, Popkin TJ. Melanin-lacking mutants of *Cryptococcus neoformans* and their virulence for mice. J Bacteriol. 1982;150:1414–21.
75. Wang Y, Aisen P, Casadevall A. *Cryptococcus neoformans* melanin and virulence: mechanism of action. Infect Immun. 1995;63: 3131–6.
76. Rittershaus PC, Kechichian TB, Allegood JC, et al. Glucosylceramide synthase is an essential regulator of pathogenicity of *Cryptococcus neoformans*. J Clin Invest. 2006;116:1651–9.
77. Garcia J, Shea J, Alvarez-Vasquez F, et al. Mathematical modeling of pathogenicity of *Cryptococcus neoformans*. Mol Syst Biol. 2008;4:183.
78. Visnegarwala F, Graviss EA, Lacke CE, et al. Acute respiratory failure associated with cryptococcosis in patients with AIDS: analysis of predictive factors. Clin Infect Dis. 1998;27:1231–7.
79. Murray RJ, Becker P, Furth P, Criner GJ. Recovery from cryptococcemia and the adult respiratory distress syndrome in the acquired immunodeficiency syndrome. Chest. 1988;93:1304–6.
80. Chang WC, Tzao C, Hsu HH, et al. Pulmonary cryptococcosis: comparison of clinical and radiographic characteristics in immunocompetent and immunocompromised patients. Chest. 2006;129: 333–40.
81. Kerkering TM, Duma RJ, Shadomy S. The evolution of pulmonary cryptococcosis: clinical implications from a study of 41 patients with and without compromising host factors. Ann Intern Med. 1981;94:611–6.
82. Pappas PG. Therapy of cryptococcal meningitis in non-HIV-infected patients. Curr Infect Dis Rep. 2001;3:365–70.
83. Baddley JW, Perfect JR, Oster RA, et al. Pulmonary cryptococcosis in patients without HIV infection: factors associated with disseminated disease. Eur J Clin Microbiol Infect Dis. 2008;27: 937–43.
84. Roebuck DJ, Fisher DA, Currie BJ. Cryptococcosis in HIV negative patients: findings on chest radiography. Thorax. 1998;53:554–7.
85. Oliveira Fde M, Severo CB, Guazzelli LS, Severo LC. *Cryptococcus gattii* fungemia: report of a case with lung and brain lesions mimicking radiological features of malignancy. Rev Inst Med Trop São Paulo. 2007;49:263–5.
86. Speed B, Dunt D. Clinical and host differences between infections with the two varieties of *Cryptococcus neoformans*. Clin Infect Dis. 1995;21:28–34.
87. Khoury MB, Godwin JD, Ravin CE, Gallis HA, Halvorsen RA, Putman CE. Thoracic cryptococcosis: immunologic competence and radiologic appearance. Am J Roentgenol. 1984;142:893–6.
88. Shirley RM, Baddley JW. Cryptococcal lung disease. Curr Opin Pulm Med. 2009;15:254–60.
89. Woodring JH, Ciporkin G, Lee C, Worm B, Woolley S. Pulmonary cryptococcosis. Semin Roentgenol. 1996;31:67–75.
90. Cameron ML, Bartlett JA, Gallis HA, Waskin HA. Manifestations of pulmonary cryptococcosis in patients with acquired immunodeficiency syndrome. Rev Infect Dis. 1991;13:64–7.
91. Meyohas MC, Roux P, Bollens D, et al. Pulmonary cryptococcosis: localized and disseminated infections in 27 patients with AIDS. Clin Infect Dis. 1995;21:628–33.
92. Saag MS, Powderly WG, Cloud GA, The NIAID Mycoses Study Group and the AIDS Clinical Trials Group, et al. Comparison of amphotericin B with fluconazole in the treatment of acute AIDS-associated cryptococcal meningitis. N Engl J Med. 1992;326:83–9.
93. van der Horst CM, Saag MS, Cloud GA, National Institute of Allergy and Infectious Diseases Mycoses Study Group and AIDS Clinical Trials Group, et al. Treatment of cryptococcal meningitis associated with the acquired immunodeficiency syndrome. N Engl J Med. 1997;337:15–21.
94. Graybill JR, Sobel J, Saag M, The NIAID Mycoses Study Group and AIDS Cooperative Treatment Groups, et al. Diagnosis and management of increased intracranial pressure in patients with AIDS and cryptococcal meningitis. Clin Infect Dis. 2000;30: 47–54.
95. Pappas PG. Managing cryptococcal meningitis is about handling the pressure. Clin Infect Dis. 2005;40:480–2.
96. Bicanic T, Brouwer AE, Meintjes G, et al. Relationship of cerebrospinal fluid pressure, fungal burden and outcome in patients with cryptococcal meningitis undergoing serial lumbar punctures. AIDS (London). 2009;23:701–6.
97. Fujita NK, Reynard M, Sapico FL, Guze LB, Edwards Jr JE. Cryptococcal intracerebral mass lesions: the role of computed tomography and nonsurgical management. Ann Intern Med. 1981; 94:382–8.
98. Charlier C, Dromer F, Leveque C, et al. Cryptococcal neuroradiological lesions correlate with severity during cryptococcal meningoencephalitis in HIV-positive patients in the HAART era. PloS. 2008;3:e1950.
99. Singh N, Lortholary O, Dromer F, et al. Central nervous system cryptococcosis in solid organ transplant recipients: clinical relevance of abnormal neuroimaging findings. Transplantation. 2008;86:647–51.
100. Mitchell DH, Sorrell TC, Allworth AM, et al. Cryptococcal disease of the CNS in immunocompetent hosts: influence of cryptococcal variety on clinical manifestations and outcome. Clin Infect Dis. 1995;20:611–6.
101. Popovich MJ, Arthur RH, Helmer E. CT of intracranial cryptococcosis. Am J Roentgenol. 1990;154:603–6.
102. Park MK, Hospenthal DR, Bennett JE. Treatment of hydrocephalus secondary to cryptococcal meningitis by use of shunting. Clin Infect Dis. 1999;28:629–33.
103. Liliang PC, Liang CL, Chang WN, Lu K, Lu CH. Use of ventriculoperitoneal shunts to treat uncontrollable intracranial hypertension in patients who have cryptococcal meningitis without hydrocephalus. Clin Infect Dis. 2002;34:E64–8.
104. Diamond RD, Bennett JE. Prognostic factors in cryptococcal meningitis. A study in 111 cases. Ann Intern Med. 1974;80:176–81.
105. Lu CH, Chang WN, Chang HW, Chuang YC. The prognostic factors of cryptococcal meningitis in HIV-negative patients. J Hosp Infect. 1999;42:313–20.
106. Powderly WG, Saag MS, Cloud GA, The NIAID AIDS Clinical Trials Group and Mycoses Study Group, et al. A controlled trial of fluconazole or amphotericin B to prevent relapse of cryptococcal meningitis in patients with the acquired immunodeficiency syndrome. N Engl J Med. 1992;326:793–8.
107. Powderly WG, Cloud GA, Dismukes WE, Saag MS. Measurement of cryptococcal antigen in serum and cerebrospinal fluid: value in the management of AIDS-associated cryptococcal meningitis. Clin Infect Dis. 1994;18:789–92.
108. Bicanic T, Wood R, Meintjes G, et al. High-dose amphotericin B with flucytosine for the treatment of cryptococcal meningitis in HIV-infected patients: a randomized trial. Clin Infect Dis. 2008;47: 123–30.
109. McNeil MM, Nash SL, Hajjeh RA, et al. Trends in mortality due to invasive mycotic diseases in the United States, 1980–1997. Clin Infect Dis. 2001;33:641–7.
110. Baer S, Baddley JW, Gnann JW, Pappas PG. Cryptococcal disease presenting as necrotizing cellulitis in transplant recipients. Transpl Infect Dis 2009.
111. Pema K, Diaz J, Guerra LG, Nabhan D, Verghese A. Disseminated cutaneous cryptococcosis. Comparison of clinical manifestations in the pre-AIDS and AIDS eras. Arch Intern Med. 1994;154: 1032–4.
112. Anderson DJ, Schmidt C, Goodman J, Pomeroy C. Cryptococcal disease presenting as cellulitis. Clin Infect Dis. 1992;14:666–72.

113. Casadevall A, Mukherjee J, Yuan R, Perfect J. Management of injuries caused by *Cryptococcus neoformans*–contaminated needles. Clin Infect Dis. 1994;19:951–3.
114. Neuville S, Dromer F, Morin O, Dupont B, Ronin O, Lortholary O. Primary cutaneous cryptococcosis: a distinct clinical entity. Clin Infect Dis. 2003;36:337–47.
115. Behrman RE, Masci JR, Nicholas P. Cryptococcal skeletal infections: case report and review. Rev Infect Dis. 1990;12:181–90.
116. Nottebart HC, McGehee RF, Utz JP. *Cryptococcus neoformans* osteomyelitis: case report of two patients. Sabouraudia. 1974;12: 127–32.
117. Stead KJ, Klugman KP, Painter ML, Koornhof HJ. Septic arthritis due to *Cryptococcus neoformans*. J Infect. 1988;17:139–45.
118. Perfect JR. Cryptococcosis. Infect Dis Clin N Am. 1989;3:77–102.
119. Braman RT. Cryptococcosis (torulosis) of prostate. Urology. 1981;17:284–5.
120. Crump JR, Elner SG, Elner VM, Kauffman CA. Cryptococcal endophthalmitis: case report and review. Clin Infect Dis. 1992;14: 1069–73.
121. Leavitt AD, Kauffman CA. Cryptococcal aortitis. Am J Med. 1988;85:108–10.
122. Staib F, Seibold M, L'Age M, et al. *Cryptococcus neoformans* in the seminal fluid of an AIDS patient. A contribution to the clinical course of cryptococcosis. Mycoses. 1989;32:171–80.
123. Larsen RA, Bozzette S, McCutchan JA, Chiu J, Leal MA, Richman DD, et al. Persistent *Cryptococcus neoformans* infection of the prostate after successful treatment of meningitis. Ann Intern Med. 1989;111:125–8.
124. Bicanic T, Meintjes G, Rebe K, et al. Immune reconstitution inflammatory syndrome in HIV-associated cryptococcal meningitis: a prospective study. J Acquir Immune Defic Syndr. 2009;51: 130–4.
125. Lortholary O, Fontanet A, Memain N, Martin A, Sitbon K, Dromer F. Incidence and risk factors of immune reconstitution inflammatory syndrome complicating HIV-associated cryptococcosis in France. AIDS (London). 2005;19:1043–9.
126. Shelburne 3rd SA, Darcourt J, White Jr AC, et al. The role of immune reconstitution inflammatory syndrome in AIDS-related *Cryptococcus neoformans* disease in the era of highly active antiretroviral therapy. Clin Infect Dis. 2005;40:1049–52.
127. Singh N, Lortholary O, Alexander BD, et al. An immune reconstitution syndrome-like illness associated with *Cryptococcus neoformans* infection in organ transplant recipients. Clin Infect Dis. 2005;40:1756–61.
128. Shelburne SA, Visnegarwala F, Darcourt J, et al. Incidence and risk factors for immune reconstitution inflammatory syndrome during highly active antiretroviral therapy. AIDS. 2005;19:399–406.
129. Singh N, Dromer F, Perfect JR, Lortholary O. Cryptococcosis in solid organ transplant recipients: current state of the science. Clin Infect Dis. 2008;47:1321–7.
130. Bennett JE, Dismukes WE, Duma RJ, et al. A comparison of amphotericin B alone and combined with flucytosine in the treatment of cryptococcal meningitis. N Engl J Med. 1979;301:126–31.
131. Aberg JA, Mundy LM, Powderly WG. Pulmonary cryptococcosis in patients without HIV infection. Chest. 1999;115:734–40.
132. Campbell CK, Payne AL, Teall AJ, Brownell A, Mackenzie DW. Cryptococcal latex antigen test positive in patient with *Trichosporon beigelii* infection. Lancet. 1985;2(8445):43–4.
133. Tarrand JJ, Guillot C, Wenglar M, Jackson J, Lajeunesse JD, Rolston KV. Clinical comparison of the resin-containing BACTEC 26 Plus and the Isolator 10 blood culturing systems. J Clin Microbiol. 1991;29:2245–9.
134. Reference method for broth dilution antifungal susceptibility testing of yeasts: approved standard, 3rd edn., M23-A3. Wayne: Clinical Laboratory Standards Institute, 2008.
135. Brandt ME, Pfaller MA, Hajjeh RA, et al. Trends in antifungal drug susceptibility of *Cryptococcus neoformans* isolates in the United States: 1992 to 1994 and 1996 to 1998. Antimicrob Agents Chemother. 2001;45:3065–9.
136. Pfaller MA, Diekema DJ, Gibbs DL, et al. Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: 10.5-year analysis of susceptibilities of noncandidal yeast species to fluconazole and voriconazole determined by CLSI standardized disk diffusion testing. J Clin Microbiol. 2009;47:117–23.
137. Torres-Rodriguez JM, Alvarado-Ramirez E, Murciano F, Sellart M. MICs and minimum fungicidal concentrations of posaconazole, voriconazole and fluconazole for *Cryptococcus neoformans* and *Cryptococcus gattii*. J Antimicrob Chemother. 2008;62:205–6.
138. Bartizal K, Gill CJ, Abruzzo GK, et al. In vitro preclinical evaluation studies with the echinocandin antifungal MK-0991 (L-743, 872). Antimicrob Agents Chemother. 1997;41:2326–32.
139. Aller AI, Martin-Mazuelos E, Lozano F, et al. Correlation of fluconazole MICs with clinical outcome in cryptococcal infection. Antimicrob Agents Chemother. 2000;44:1544–8.
140. Armengou A, Porcar C, Mascaro J, Garcia-Bragado F. Possible development of resistance to fluconazole during suppressive therapy for AIDS-associated cryptococcal meningitis. Clin Infect Dis. 1996;23:1337–8.
141. Witt MD, Lewis RJ, Larsen RA, et al. Identification of patients with acute AIDS-associated cryptococcal meningitis who can be effectively treated with fluconazole: the role of antifungal susceptibility testing. Clin Infect Dis. 1996;22:322–8.
142. Perfect JR, Dismukes WE, Dromer F, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the Infectious Disease Society of America. Clin Infect Dis. 2010;50:291–322.
143. Hospenthal DR, Bennett JE. Flucytosine monotherapy for cryptococcosis. Clin Infect Dis. 1998;27:260–4.
144. Harrison TS. Amphotericin B plus fluconazole for HIV-associated cryptococcal meningitis. Clin Infect Dis. 2009;48:1784–6.
145. Pappas PG, Chetchotisakd P, Larsen RA, et al. A phase II randomized trial of amphotericin B alone or combined with fluconazole in the treatment of HIV-associated cryptococcal meningitis. Clin Infect Dis. 2009;48:1775–83.
146. Dromer F, Mathoulin S, Dupont B, Brugiere O, Letenneur L, French Cryptococcosis Study Group. Comparison of the efficacy of amphotericin B and fluconazole in the treatment of cryptococcosis in human immunodeficiency virus-negative patients: retrospective analysis of 83 cases. Clin Infect Dis. 1996;22 Suppl 2:S154–60.
147. Nadrous HF, Antonios VS, Terrell CL, Ryu JH. Pulmonary cryptococcosis in nonimmunocompromised patients. Chest. 2003;124: 2143–7.
148. Jarvis JN, Harrison TS. Pulmonary cryptococcosis. Semin Respir Crit Care Med. 2008;29:141–50.
149. Yamaguchi H, Ikemoto H, Watanabe K, Ito A, Hara K, Kohno S. Fluconazole monotherapy for cryptococcosis in non-AIDS patients. Eur J Clin Microbiol Infect Dis. 1996;15:787–92.
150. Denning DW, Tucker RM, Hanson LH, Hamilton JR, Stevens DA. Itraconazole therapy for cryptococcal meningitis and cryptococcosis. Arch Intern Med. 1989;149:2301–8.
151. Raad II, Graybill JR, Bustamante AB, et al. Safety of long-term oral posaconazole use in the treatment of refractory invasive fungal infections. Clin Infect Dis. 2006;42:1726–34.
152. Perfect JR, Marr KA, Walsh TJ, et al. Voriconazole treatment for less-common, emerging, or refractory fungal infections. Clin Infect Dis. 2003;36:1122–31.
153. Sharkey PK, Graybill JR, Johnson ES, et al. Amphotericin B lipid complex compared with amphotericin B in the treatment of cryptococcal meningitis in patients with AIDS. Clin Infect Dis. 1996;22:315–21.

154. Coker RJ, Viviani M, Gazzard BG, et al. Treatment of cryptococcosis with liposomal amphotericin B (AmBisome) in 23 patients with AIDS. AIDS (London, England). 1993;7:829–35.
155. Leenders AC, Reiss P, Portegies P, et al. Liposomal amphotericin B (AmBisome) compared with amphotericin B both followed by oral fluconazole in the treatment of AIDS-associated cryptococcal meningitis. AIDS (London). 1997;11:1463–71.
156. Hammerman KJ, Powell KE, Christianson CS, et al. Pulmonary cryptococcosis: clinical forms and treatment. A Center for Disease Control Cooperative Mycoses study. Am Rev Respir Dis. 1973;108:1116–23.
157. Husain S, Wagener MM, Singh N. *Cryptococcus neoformans* infection in organ transplant recipients: variables influencing clinical characteristics and outcome. Emerg Infect Dis. 2001;7:375–81.
158. Hospenthal DR, Bennett JE. Persistence of cryptococcomas on neuroimaging. Clin Infect Dis. 2000;31:1303–6.
159. Larsen RA, Bozzette SA, Jones BE, et al. Fluconazole combined with flucytosine for treatment of cryptococcal meningitis in patients with AIDS. Clin Infect Dis. 1994;19:741–5.
160. Mayanja-Kizza H, Oishi K, Mitarai S, et al. Combination therapy with fluconazole and flucytosine for cryptococcal meningitis in Ugandan patients with AIDS. Clin Infect Dis. 1998;26:1362–6.
161. Menichetti F, Fiorio M, Tosti A, et al. High-dose fluconazole therapy for cryptococcal meningitis in patients with AIDS. Clin Infect Dis. 1996;22:838–40.
162. Haubrich RH, Haghighat D, Bozzette SA, Tilles J, McCutchan JA, The California Collaborative Treatment Group. High-dose fluconazole for treatment of cryptococcal disease in patients with human immunodeficiency virus infection. J Infect Dis. 1994;170:238–42.
163. de Gans J, Portegies P, Tiessens G, et al. Itraconazole compared with amphotericin B plus flucytosine in AIDS patients with cryptococcal meningitis. AIDS (London). 1992;6:185–90.
164. Bozzette SA, Larsen RA, Chiu J, California Collaborative Treatment Group, et al. A placebo-controlled trial of maintenance therapy with fluconazole after treatment of cryptococcal meningitis in the acquired immunodeficiency syndrome. N Engl J Med. 1991;324:580–4.
165. Saag MS, Cloud GA, Graybill JR, National Institute of Allergy and Infectious Diseases Mycoses Study Group, et al. A comparison of itraconazole versus fluconazole as maintenance therapy for AIDS-associated cryptococcal meningitis. Clin Infect Dis. 1999;28:291–6.
166. Kaplan JE, Benson C, Holmes KH, Brooks JT, Pau A, Masur H. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. MMWR. 2009;58:1–207.
167. Mussini C, Pezzotti P, Miro JM, et al. Discontinuation of maintenance therapy for cryptococcal meningitis in patients with AIDS treated with highly active antiretroviral therapy: an international observational study. Clin Infect Dis. 2004;38:565–71.
168. Vibhagool A, Sungkanuparph S, Mootsikapun P, et al. Discontinuation of secondary prophylaxis for cryptococcal meningitis in human immunodeficiency virus-infected patients treated with highly active antiretroviral therapy: a prospective, multicenter, randomized study. Clin Infect Dis. 2003;36:1329–31.
169. Bicanic T, Meintjes G, Wood R, et al. Fungal burden, early fungicidal activity, and outcome in cryptococcal meningitis in antiretroviral-naive or antiretroviral-experienced patients treated with amphotericin B or fluconazole. Clin Infect Dis. 2007;45:76–80.
170. Chuck SL, Sande MA. Infections with *Cryptococcus neoformans* in the acquired immunodeficiency syndrome. N Engl J Med. 1989;321:794–9.
171. Larsen RA, Leal MA, Chan LS. Fluconazole compared with amphotericin B plus flucytosine for cryptococcal meningitis in AIDS. A randomized trial. Ann Intern Med. 1990;113:183–7.
172. Brouwer AE, Rajanuwong A, Chierakul W, et al. Combination antifungal therapies for HIV-associated cryptococcal meningitis: a randomised trial. Lancet. 2004;363:1764–7.
173. Dromer F, Bernede-Bauduin C, Guillemot D, Lortholary O. Major role for amphotericin B-flucytosine combination in severe cryptococcosis. PloS. 2008;3:2870.
174. Baddour LM, Perfect JR, Ostrosky-Zeichner L. Successful use of amphotericin B lipid complex in the treatment of cryptococcosis. Clin Infect Dis. 2005;40 Suppl 6:S409–13.
175. Longley N, Muzoora C, Taseera K, et al. Dose response effect of high-dose fluconazole for HIV-associated cryptococcal meningitis in southwestern Uganda. Clin Infect Dis. 2008;47:1556–61.
176. Aberg JA, Price RW, Heeren DM, Bredt B. A pilot study of the discontinuation of antifungal therapy for disseminated cryptococcal disease in patients with acquired immunodeficiency syndrome, following immunologic response to antiretroviral therapy. J Infect Dis. 2002;185:1179–82.
177. Zolopa A, Andersen J, Komarow L, et al. Immediate vs. deferred ART in the setting of a randomized strategy trial ACTG A5164. 15th Conference on Retroviruses and Opportunistic Infections. February, 2008. Boston. Abstract 142.
178. Newton PN, le Thai H, Tip NQ, et al. A randomized, double-blind, placebo-controlled trial of acetazolamide for the treatment of elevated intracranial pressure in cryptococcal meningitis. Clin Infect Dis. 2002;35:769–72.
179. McKinsey DS, Wheat LJ, Cloud GA, National Institute of Allergy and Infectious Diseases Mycoses Study Group, et al. Itraconazole prophylaxis for fungal infections in patients with advanced human immunodeficiency virus infection: randomized, placebo-controlled, double-blind study. Clin Infect Dis. 1999;28:1049–56.
180. Powderly WG, Finkelstein D, Feinberg J, NIAID AIDS Clinical Trials Group, et al. A randomized trial comparing fluconazole with clotrimazole troches for the prevention of fungal infections in patients with advanced human immunodeficiency virus infection. N Engl J Med. 1995;332:700–5.
181. Jarvis JN, Lawn SD, Vogt M, Bangani N, Wood R, Harrison TS. Screening for cryptococcal antigenemia in patients accessing an antiretroviral treatment program in South Africa. Clin Infect Dis. 2009;48:856–62.
182. Tassie JM, Pepper L, Fogg C, et al. Systematic screening of cryptococcal antigenemia in HIV-positive adults in Uganda. J Acquir Immune Defic Syndr. 2003;33:411–2.
183. Nelson MR, Bower M, Smith D, Reed C, Shanson D, Gazzard B. The value of serum cryptococcal antigen in the diagnosis of cryptococcal infection in patients infected with the human immunodeficiency virus. J Infect. 1990;21:175–81.
184. Desmet P, Kayembe KD, De Vroey C. The value of cryptococcal serum antigen screening among HIV-positive/AIDS patients in Kinshasa, Zaire. AIDS (London). 1989;3:77–8.
185. Liechty CA, Solberg P, Were W, et al. Asymptomatic serum cryptococcal antigenemia and early mortality during antiretroviral therapy in rural Uganda. Trop Med Int Health. 2007;12:929–35.
186. Micol R, Lortholary O, Sar B, et al. Prevalence, determinants of positivity, and clinical utility of cryptococcal antigenemia in Cambodian HIV-infected patients. J Acquir Immune Defic Syndr. 2007;45:555–9.
187. French N, Gray K, Watera C, et al. Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. AIDS (London). 2002;16:1031–8.