HHS Public Access

Author manuscript

Infect Dis Clin North Am. Author manuscript; available in PMC 2018 February 12.

Published in final edited form as:

Infect Dis Clin North Am. 2016 March ; 30(1): 179–206. doi:10.1016/j.idc.2015.10.006.

Cryptococcosis

Eileen K. Maziarz, MD[*] and **John R. Perfect, MD**

Division of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, DUMC Box 102359, 315 Trent Drive, Durham, NC 27710, USA

Keywords

Cryptococcosis; Opportunistic mycoses; HIV/AIDS; Solid organ transplantation (SOT); Central nervous system (CNS) infection; Immune reconstitution inflammatory syndrome (IRIS)

INTRODUCTION

Cryptococcosis is an infectious disease with worldwide distribution and wide array of clinical presentations caused by pathogenic encapsulated yeasts in the genus *Cryptococcus*. Currently, there are 2 species of *Cryptococcus* that commonly cause disease in humans: *Cryptococcus neoformans* and *Cryptococcus gattii*. *C neoformans* was first identified as a human pathogen in the late 19th century, but was not recognized as a common cause of human disease until the late 1970s.[1,2] Over the last several decades, as vulnerable populations have expanded, cryptococcal meningitis became an infection of global importance, with up to 1 million new infections annually and significant attributable morbidity and mortality, especially among patients with human immunodeficiency virus (HIV) infection and AIDS.[3] Although *C neoformans and C gattii* share many features of a highly evolved, environmentally savvy yeast, there are important species- and strain-specific differences with respect to geographic distribution, environmental niches, host predilection, and clinical manifestations that should be emphasized. As molecular techniques of identification have evolved, we have gained further insight into the pathobiology of these encapsulated yeasts, and their capacity to adapt to environmental pressures, exploit new geographic environments, and cause disease in both immunocompromised and apparently immunocompetent hosts.[4] Despite increased availability of and success with antiretroviral therapy (ART), the worldwide burden of and mortality associated cryptococcal disease remains unacceptably high, and novel strategies of screening and preemptive therapy offer great promise at making a sustained and much needed impact on this sugarcoated opportunistic mycosis.

[*]**Corresponding author.** eileen.maziarz@dm.duke.edu.

Disclosure Statement: Dr J.R. Perfect is a Principal Investigator for the following companies: Amplyx, Astellas, Cidara, Merck, Pfizer, Schering-Plough, Tokoyama, and Viamet.

THE PATHOGENS: CRYPTOCOCCUS NEOFORMANS AND CRYPTOCOCCUS GATTII

Cryptococcus is a genus of basidiomycetous fungi with more than 30 species ubiquitously distributed in the environment. There are only 2 species commonly known to cause human disease, *C neoformans* and *C gattii*. The epidemiology of *C neoformans* is well-characterized and this organism causes disease in both immunocompromised and apparently immunocompetent hosts. *C gattii*, conversely, has historically been regarded as a pathogen of apparently immunocompetent patients. However, preexisting conditions and immunocompromised states, including subclinical immune defects, are also reported as risk factors for infection with this species.[5–8] These species differences in clinical presentation may be primarily determined by variable host predilections, but may also be better characterized as we further our understanding of molecular subtypes.[9–12]

Historically, the genus was further classified into 3 varieties, 5 serotypes (based on structural differences in the polysaccharide capsule), and 8 molecular subtypes (Table 1). Molecular methods of identification have enhanced our appreciation for the significant genetic diversity among the *C gattii–C neoformans* complex and have called into question the current 2 species classification system. Recent proposed taxonomy changes based on the understanding of molecular studies have divided the pathogenic cryptococcal species from their classic divisions into better-defined molecular and genetic divisions. At present, the following divisions have been proposed: *C neoformans* var. *grubii* (serotype A) with 3 genotypes (VNI, VNII, VNB); *C neoformans* var. *neoformans* (serotype D or VNIV); and 5 other cryptic species, *C gattii*, *C bacillisporus*, *C deuterogattii*, *C tetragattii*, and *C decagattii* (serotypes B/C or VGI-IV).[13] Phylogenetic analyses, combined with recognized heterogeneity with respect to virulence, host preference, and antifungal susceptibility do provide evidence to support further taxonomic classification into a 7-species/4 hybrid species scheme (Table 2). The molecular taxonomy of cryptococcal species is a vibrant area of evolution that has allowed for a greater understanding of specific strain characteristics, including fitness and predilection for certain environmental niches[13]; clinical correlations have yet to match this molecular precision, however, and for this review we will tend to lump the yeasts into their historical species designations, *C neoformans* and *C gattii*.

Approximately 95% of cryptococcal infections are caused by *C neoformans* (serotype A) strains with the remaining 4% to 5% of infections caused by *C neoformans* (serotype D) or *C gattii* (serotypes B/C strains). Whereas *C neoformans* var. *grubii* (serotype A) is found worldwide, *C neoformans* var *neoformans* (serotype D) is primarily observed in European countries and *C gattii* has historically been geographically restricted to tropical and subtropical regions, such as southern California, Hawaii, Brazil, Australia, Southeast Asia, and central Africa. More recently, *C gattii* has been identified in temperate climates such as Vancouver Island and the Pacific Northwest region of the United States and parts of Europe, suggesting an ecological shift possibly related to global temperature and moisture changes.[4,10–12] Although *C gattii* causes up to 15% of all cases of cryptococcosis in Australia and New Zealand, *C neoformans* remains the predominant species even in these endemic areas.[14] In certain areas of Africa around Botswana, where *C neoformans and C gattii* live

together in the environment, active sexual recombination has been reported.[15] Although outbreaks of cryptococcosis are ongoing among immunocompromised populations worldwide, to date only *C gattii* strains have been reported to produce a geographically defined outbreak of disease.[4]

C neoformans is found throughout the world in association with excreta from certain birds such as pigeons,[16] environmental scavengers such as ameba and sowbugs,[17,18] and in a variety of tree species in their hollows. *C gattii* is commonly associated with several species of eucalyptus trees in tropical and subtropical climates.[19] However, recently as it has emerged as an important pathogen capable of widespread outbreaks within new geographic niches including British Columbia and the Pacific Northwest United States,[4,10–12] it has been associated with temperate trees, such as firs and oaks.[9,20–22]

The life cycle of *Cryptococcus* involves both asexual and sexual forms.[23] The asexual form is the haploid encapsulated yeast that reproduces by mitosis with narrow-based budding and is found in clinical and environmental specimens. The sexual state is observed at present under certain laboratory conditions, resulting in meiosis between 2 mating types (MATa and MATα) to form clamp connections, basidia and basidiospores. The α mating type strains represent the vast majority of clinical and environmental isolates, probably related to their ability to produce haploid fruiting. Even same sex mating between 2 strains of the same type (MATα–MATα) does occur and is thought to produce the infectious spores that cause human infection.[24,25] This nonclassical mating between 2 α–α strains allows for further genetic diversity and is implicated in the production of hypervirulent, clonal strains responsible for the *C gattii* outbreak on Vancouver Island, suggesting that such mechanisms may confer the yeast the ability to exploit new geographic niches.[26,27] Furthermore, there are locations in Botswana where there are equal proportions of MATα and MATa isolates in both environmental and clinical populations, providing evidence that sexual recombination remains active even with the spread worldwide of relatively clonal strains.[15,28]

EPIDEMIOLOGY AND RISK FACTORS

Cryptococcosis was considered an uncommon infection before the AIDS pandemic; however, it was an awakening mycosis giant in the 1970s because it was associated with malignancy, organ transplantation, and certain immunosuppressive treatments. The incidence of disease increased significantly in the mid 1980s, with HIV/AIDS accounting for more than 80% of cryptococcosis cases worldwide.[29–31] Cryptococcal meningitis preferentially occurs in persons with impaired cell-mediated immunity and is a major AIDS-related opportunistic infection as the $CD4^+$ cell count falls below 100 cells/μL. With widespread implementation of successful antiretroviral therapy (ART), the incidence of HIV-associated cryptococcosis has decreased significantly in most developed nations, although the incidence in other at-risk populations has not changed (Table 3).[32] Furthermore, the prevalence of and morbidity and mortality associated with cryptococcal meningitis remain unacceptably high in settings where access to ART and other necessary health care resources are limited, specifically sub-Saharan Africa and parts of Asia. In fact, mortality peaked at approximately 600,000 deaths per year in the first decade of the 21st century; even today, it is likely that cryptococcal meningitis–related deaths approach several

hundred thousand per year.[3] Although both *C neoformans* and *C gattii* can also cause disease in apparently immunocompetent hosts, the percentage of infections owing to *C gattii* in such patients is significantly higher than for *C neoformans*.

Pathogenesis and Host Immunity

Cryptococcal infection occurs primarily by inhalation of the infectious propagules (either poorly encapsulated yeast cells or basidiospores) from environmental reservoirs with deposition into pulmonary alveoli. Traumatic inoculation into tissues has been described[33] and may occur infrequently. The yeast may potentially enter via the gastrointestinal tract, although this entry is less consistent. Primary pulmonary infection is generally thought to be asymptomatic or minimally symptomatic despite high rates of serologic reactivity in children in certain urban settings.[34] Clearance of the infection by the host may occur. However, in many individuals, after yeasts are deposited in alveoli, they encounter alveolar macrophages, which play a central role in the immune response.[35] Host response to cryptococcal infection primarily involves a helper T cell response with cytokines including tumor necrosis factor (TNF), interferon-γ, and interleukin-2, resulting in granulomatous inflammation.[36] In many circumstances, this yeast will establish a latent infection within phagolysosome, with dormant (yet viable) yeasts within the thoracic lymph nodes or a pulmonary granuloma that can persist in an asymptomatic individual for years. When local immunity is suppressed, the yeast can grow and disseminate outside these pulmonary lymph node complexes similar to the pathophysiology that is observed in cases of reactivation tuberculosis or histoplasmosis.[31,37] In some hosts, *C gattii* disease seems to be more likely than *C neoformans* disease to present as a progressive granulomatous pulmonary infection, but less likely to disseminate to the central nervous system (CNS). This general observation has been made in human outbreaks and characterized in mouse models, but there remains substantial overlap between species.[12,31,38] In a patient with severely compromised cellular immunity, the yeasts reactivate and can proliferate at the site of initial infection and can disseminate within phagocytes or as yeast cells and gain access to other body sites.[39] Both direct invasion of the blood–brain barrier via transcytosis of free yeast forms through a series of mechanisms between yeast and host factors[40] and/or transport via macrophages into the CNS (the “Trojan horse” mechanism) seem to occur.[41–43] Whether certain immune states permit additional body sites of latency (eg, the CNS or prostate) have not yet been elucidated fully.

Advances in the molecular biology of *Cryptococcus* have confirmed multiple yeast virulence factors.[44] The 3 classical and prominent virulence factors of *C neoformans* include capsule formation, melanin pigment production, and thermotolerance.[23,36] The prominent antiphagocytic polysaccharide capsule, which is composed of glucuronoxylomannan, is unique to *Cryptococcus* species and is considered an essential virulence factor that has multiple effects on host immunity and can increase in size with exposure to body tissues and fluids.[45,46] In addition, *C neoformans* possesses an enzyme that catalyzes the conversion of diphenolic compounds to form melanin, which, when expressed, may have a biological role to protect the yeasts from host oxidative stresses and which may partially explain the organism’s neurotropism into sites with high concentrations of the diphenolic catecholamines. Finally, the ability to grow at 37°C is a basic part of the virulence composite

for most pathogenic fungi in humans including *Cryptococcus*, and molecular studies have linked high temperature growth with multiple signaling pathways and enzymes that this yeast has acquired or adapted to over time to retain or enhance its mammalian pathogenicity. Other virulence factors include phospholipase and urease production and multiple enzymes associated with protection against oxidative stresses, conferring survival within the phagolysosome.[44] It is estimated that more than 100 genes are important for optimal fitness of the yeast in mammalian hosts. The yeast has even adapted sophisticated mechanisms to escape the intracellular environment by modifying the permeability of the phagosome membrane and via nonlytic exocytosis (vomocytosis), allowing cell-to-cell or host compartment transfer of yeast ant its virulence factors without damage to the host macrophages.[47,48]

The many factors in the immunologic responses to *Cryptococcus* cannot be covered completely in this review, but several observations can be made. First, exposure is frequent and the healthy immunocompetent individual is generally resistant to cryptococcal disease. In fact, even in this group, some apparently normal hosts with cryptococcosis have been found to possess anti-granulocyte macrophage colony stimulating factor antibodies as a potential immune defect.[7,8] Second, the effective immune response is through a helper T cell–supported reaction and anything that weakens it may let cryptococci survive and thrive. This includes destruction of $CD4^+$ cells by HIV, reduction of TNF activity by anti-TNF inhibitors, or the multifaceted immune suppressant effect of corticosteroids. From activated macrophages and not alternative macrophages to the development of protective antibodies over nonprotective antibodies, immunity changes over the course of cryptococcal infections. In fact, even some of our protective host mechanisms might be used against us as surfactant D may be coopted by *Cryptococcus* to gain entry into the lung.[49] Clearly, cryptococcosis emphasizes the Goldilocks paradigm of immunity. It produces disease when immunity is too little or too much, but when the human host immunity is just right, disease does not appear.

CLINICAL MANIFESTATIONS

C neoformans and *C gattii* have a major predilection for establishing clinical disease in the lungs and CNS. Other less frequent body sites of infection include skin, prostate, eyes, and bone/joints. However, it should be emphasized that this yeast can widely disseminate and infect most organs in severely immunosuppressed patients and thus has the ability to appear at any human body site.

Pulmonary Infection

The respiratory tract serves as the most important portal of entry for *Cryptococcus*. Clinical manifestations of pulmonary cryptococcosis range from asymptomatic colonization of the airways or a simple pulmonary nodule on a chest radiograph to life-threatening pneumonia with the presence of an acute respiratory distress syndrome.[50,51] In a normal host, asymptomatic, isolated pulmonary infection can occur in about one-third of patients and can be identified simply by an abnormal chest radiograph. In fact, the most common radiologic findings of cryptococcosis include well-defined single or multiple noncalcified nodules and pulmonary infiltrates (Fig. 1), although pleural effusions, hilar lymphadenopathy, and lung

cavitation may also be observed. Patients with pulmonary cryptococcosis can present acutely with symptoms of pneumonia.[50] For example, in the recent outbreak of *C gattii* infections in Vancouver Island area, several cases of severe, symptomatic pulmonary cryptococcosis in apparently immunocompetent individuals occurred.[12] In an immunocompromised patient, however, cryptococcal pneumonia is usually symptomatic and in some cases can progress rapidly to acute respiratory distress syndrome, even in the absence of CNS involvement. Pulmonary involvement ranges from 10% to 55% of patients with AIDS-associated cryptococcal meningoencephalitis, although CNS symptoms usually predominate the clinical picture.[51]

Serum cryptococcal polysaccharide antigen testing is usually negative in cases of true isolated pulmonary cryptococcosis, but at times can be positive in the absence of CNS involvement or other apparent sites of infection. In immunocompromised individuals with *Cryptococcus* isolated from the lung or other sterile body site, however, a lumbar puncture to rule out CNS disease should be considered regardless of a patient's symptoms or serum antigen titer results. The only setting wherein a screening lumbar puncture may not necessarily be required is a patient with *Cryptococcus* isolated from the lung in the apparently immunocompetent patient without referable CNS symptoms and disease that clinically seems to be limited to the lungs.

Central Nervous System Infection

Clinical manifestations of CNS cryptococcosis include a myriad of signs and symptoms, such as headache, fever, cranial neuropathies, altered mentation, lethargy, memory loss, and signs of meningeal irritation.[2,30,31] Symptoms usually develop over a period of several weeks. However, on some occasions, patients present more acutely or lack typical features, such as headache. In severely immunocompromised, HIV-infected patients with CNS cryptococcosis, the burden of fungal organisms is usually high and can reach levels of more than 1 million yeasts per milliliter of cerebrospinal fluid (CSF). These patients may consequently have a shorter onset of signs and symptoms, greater CSF polysaccharide antigen titers, and higher intracranial pressures than other more immunocompetent individuals.

Although disease severity is determined primarily by host immune factors, different species and/or strains of *Cryptococcus* may produce unique clinical manifestations, which can have implications for management. For instance, in certain areas of the world, *C gattii* has been observed to cause cerebral cryptococcomas and/or obstructive hydrocephalus with or without large pulmonary mass lesions more frequently than *C neoformans*.[12,52,53] These patients with parenchymal brain involvement may have a high intracranial pressure and present with cranial neuropathies. In such patients, who have been observed to respond poorly to antifungal therapy, early neurosurgical intervention to control pressure or ensure a correct diagnosis and longer antifungal treatment courses may be required for a successful outcome.[9,54]

Skin Infection

Cutaneous infections are the third most common clinical manifestations of cryptococcosis and patients can present with a variety of skin lesions. Lesions are often indistinguishable from those owing to other infections; as such, a skin biopsy with culture and histopathology are absolutely essential for definitive diagnosis. Primary cutaneous cryptococcosis is very rare and is usually associated with skin injury and direct inoculation of the yeasts[33]; thus, the appearance of cutaneous lesions usually heralds the presence of disseminated infection. Solid organ transplant recipients on tacrolimus seem to be more likely to develop skin, soft tissue, and osteoarticular infections owing to *Cryptococcus*.[55] Tacrolimus acts on the temperature signaling molecule calcineurin in *Cryptococcus* and has anticryptococcal activity at high temperatures, but it loses this direct antifungal activity as environmental temperatures decrease; this may in part explain the increased frequency of cutaneous lesions in patients receiving calcineurin inhibitors.[56]

Prostate Infection

The prostate is not a rare site for cryptococcal infection, but prostatic cryptococcosis is usually asymptomatic. For instance, latent *C neoformans* infection has been recognized to disseminate in the bloodstream during urologic surgery on the prostate for other indications.[57] The prostate gland may thus serve as an important reservoir for disease relapse in patients with a high fungal tissue burden.[58] Cultures of urine or seminal fluid may still be positive for *Cryptococcus* after initial antifungal treatment of cryptococcal meningitis in poorly controlled AIDS patients,[59] strongly supporting the need for prolonged antifungal treatment to eradicate infection in sanctuary sites in these severely immunocompromised patients.

Eye Infection

In early reports of cryptococcal meningitis before the AIDS epidemic, ocular signs and symptoms were noted in a substantial proportion of cases,[60] such as ocular palsies and papilledema. Several other ocular manifestations of cryptococcosis have been identified, including extensive retinal disease with or without vitritis, which can lead to irreversible blindness.[61] Visual loss may be owing to optic nerve infiltration by yeasts or vascular compromise from intracranial hypertension. The former process results in rapid visual loss with limited effective treatments, whereas the latter phenomenon results in more gradual visual loss and can be interrupted with aggressive management of increased intracranial pressure.

Infection at Other Body Sites

C neoformans can cause disease in essentially any organ of the human body. In fact, the first identification of this fungus from a clinical specimen was from a patient with tibial osteomyelitis in the 19th century.[1] Bone involvement of cryptococcosis typically presents as circumscribed osteolytic lesions in any bone of the body, but most commonly the vertebrae, and cryptococcal osteomyelitis has been associated with underlying sarcoidosis.[62] Bone marrow infiltration can be observed in severely immunocompromised hosts. Fungal peritonitis[63] and cryptococcuria are also reported in several case series. An appreciation for this yeast's protean clinical manifestations is essential, both at the time of initial diagnosis,

as well as when immune defects are restored during treatment and immune restoration phenomena can present.

Immune Reconstitution Inflammatory Syndrome

Restoration of pathogen-specific immunity can result in a phenomenon known as the immune reconstitution inflammatory syndrome (IRIS), an entity that can occur before ("unmasking IRIS") or during ("paradoxic IRIS") antifungal therapy. Cryptococcal IRIS is best characterized in HIV-infected patients with CNS infection and is associated with significant morbidity and mortality.[64–76] In addition, IRIS is estimated to occur in 5% to 11% of solid organ transplant recipients with cryptococcal infection and is associated with increased risk of allograft failure[77–83] and may also be observed in non-HIV, nontransplant patients.[84] Proposed criteria for IRIS in HIV-associated disease include onset of symptoms within 12 months of ART initiation (with concomitant $CD4^+$ recovery).[85] These criteria are imprecise and do not address all populations at risk (Box 1). As such, it is incumbent upon the treating provider to have a high level of suspicion for this entity, as opposed to alternative diagnoses, which include progressive infection (from inadequate antifungal therapy, direct antifungal drug resistance, or persistent immune deficits), coinfection with other opportunistic infections, malignancy, or drug toxicity.

Cryptococcal IRIS is thought to represent a dysregulated reversal of a Th2 (anti-inflammatory) to a strong helper T cell (pro-inflammatory) immune response in the setting of immune recovery.[86] Multiple factors are thought to be associated with future IRIS episodes, including high yeast burden at baseline, ineffective host immune response to initial infection, and rapid restoration of immunity.[67,73] Host immune responses in various compartments may not be uniform and are likely influenced by baseline parameters at the site.[87] Differences in baseline CSF cytokine and chemokine expression are thought to facilitate the development of cryptococcal IRIS, potentially via myeloid cell trafficking to the CNS and, consequently, production of excessive inflammation.[88,89] In fact, evidence of increased macrophage activation and linked CSF pleocytosis have been observed in patients receiving early ART and may mediate increased mortality, even before recognition of the clinical syndrome of IRIS.[87]

Clinical features of cryptococcal IRIS are similar to active cryptococcal infection itself, most commonly presenting as CNS disease, although lymphadenitis, pneumonitis, multifocal disease, soft tissue involvement, and mediastinitis have all been reported.[85,90] Meningeal disease is the most serious presentation.[85] A hallmark finding is suppurative or necrotic granulomatous inflammation with yeast forms seen on histopathology of infected tissues despite negative cultures.[77,80,90,91] Despite changes in inflammatory markers, there are no reliably specific diagnostic tests for IRIS, and establishing the diagnosis presents a considerable clinical challenge, especially with atypical presentations or manifestations at distant sites.[69,92] CSF opening pressure and white blood cell count[67,68,73] at the time of an IRIS event are significantly higher than baseline values for individual patients, which combined with negative cultures, may help to distinguish IRIS from relapsed infection.[70]

Management of cryptococcal IRIS is largely based on expert opinion.[93] First, ensuring the efficacy of antifungal therapy is essential[94,95]; in the absence of disease relapse or direct

antifungal drug resistance, modification of antimicrobial therapy is generally not indicated.[93] A significant proportion of minor cases simply improve without specific treatment.[65,66,76] Corticosteroids have been shown to decrease the need for hospitalization and improve short-term quality of life and functional status in paradoxic tuberculosis-associated IRIS.[96] Although steroids may be essential in treating a serious life-threatening CNS IRIS episode owing to *Cryptococcus*, they should not be used for prevention of IRIS or to control CNS pressure, and may be harmful in some cases.[97] Immunomodulatory agents including those with anti–TNF-α activity have been used in cases of steroid-refractory IRIS.[65,98–101] Other strategies, including therapeutic lumbar drainage for intracranial hypertension[93,102] and, at times, surgical drainage of suppurative lymph nodes,[86,91] are important adjunctive measures that may be considered in severe disease. Continuation of ART in the setting of IRIS is generally recommended and has been performed safely.[66,71,92,103,104]

LABORATORY DIAGNOSIS

Definitive diagnosis of cryptococcosis is made by isolation of *Cryptococcus* from a clinical specimen or direct detection of the fungus by means of India ink staining of body fluids. There are several other methods used for the diagnosis of cryptococcosis, including histopathology of infected tissues and serologic methods. Molecular methods, although available and extensively used for research purposes, are not used currently in routine clinical practice.

Direct Examination/India Ink

The most rapid method for diagnosis of cryptococcal meningitis is direct microscopic examination for encapsulated yeasts by India ink preparation of CSF. *Cryptococcus* can be visualized as a globular, encapsulated yeast cell with or without budding, ranging in size from 5 to 20 μm in diameter (Fig. 2). The sensitivity of India ink staining of CSF depends on fungal burden and is reported to be 30% to 50% in non–AIDS-related cryptococcal meningitis and up to 80% in AIDS-related disease. False positives can result from intact lymphocytes, other tissue cells and nonviable yeast forms, which further limits the diagnostic utility of direct microscopy of CSF for cryptococcal meningitis.[105]

Culture and Identification

Cryptococcus can be cultured readily from biologic samples such as CSF, sputum, and skin biopsy on routine fungal and bacterial culture media. In adults with HIV-associated cryptococcal meningitis, CSF and blood cultures are positive in up to 90% and 70% of patients, respectively (reviewed in[106]). Colonies are usually observed on solid agar plates after 48 to 72 hours incubation at 30°C to 35°C in aerobic conditions and will appear as opaque, white-to-cream colonies that may turn orange-tan or brown after prolonged incubation. The mucoid appearance of the colony is related to the capsule size around the yeasts. Despite relatively rapid growth for most strains, cultures should be held for up to 4 weeks, particularly for patients receiving antifungal treatment.

Cytology and Histopathology

Cryptococcus can be identified by histologic staining of tissues from the lung, skin, bone marrow, brain, and other organs.[107] Histopathologic staining and cytology of centrifuged CSF sediment and other bodily fluids are more sensitive than the India ink staining method.[108–111] The organism is observed as a yeast that reproduces by narrow-based budding. The yeast is best identified by special stains that label the polysaccharide capsule including mucicarmine, periodic acid-Schiff, and Alcian blue stains.[2] The Fontana–Masson stain identifies melanin in the yeast cell wall. Other fungal stains such as Calcofluor, which binds fungal chitin, or Gomori methenamine silver, which stains the fungal cell wall, are also used to identify the organism from clinical specimens.[2,109]

Serology

The diagnosis of cryptococcosis improved significantly with the development of serologic tests for the cryptococcal polysaccharide capsular antigen (CrAg), which is shed during infection. Latex agglutination and enzyme immunoassay techniques have been available widely (using both serum and CSF), the former of which had been the most commonly used methodology until recently, with overall sensitivities and specificities of 93% to 100% and 93% to 98%, respectively.[112,113] False-positive results of latex agglutination testing usually have initial reciprocal titers of 8 or less,[112] whereas false negatives can be seen owing to a prozone effect in the setting of extremely high antigen titers, which can be overcome with dilution.[114] Low fungal burden, as in chronic low-grade meningitis or in the very early stages of infection, and improper specimen storage can also cause false-negative results in latex agglutination tests.[115] Recently, a lateral flow assay was approved for use in serum and CSF, with sensitivity and specificity of greater than 98% in both specimen types (including whole blood from finger stick samples) and sensitivity of 85% in urine.[116–123] The semiquantitative test offers many advantages over the other serologic methods, including rapid turnaround (approximately 15 minutes), minimal requirements for laboratory infrastructure, stability at room temperature, low cost, and wider capture of *C gattii* polysaccharides.[116] Combined with these advantages, the assay's excellent performance across a broad range of clinical settings, including settings with low burden of HIV infection and high rates of *C gattii* infection,[100–104] make it an attractive option for point-of-care testing in both resource-available and resource-limited settings.[116,117,124]

Baseline cryptococcal polysaccharide antigen titers in serum and CSF correlate with fungal burden and carry prognostic significance in patients with cryptococcal meningitis.[122,125,126] However, there is limited value in serial monitoring of antigen titers acutely in assessing treatment response, because the kinetics of antigen clearance is a slower and less predictable marker of treatment response than quantitative culture.[122,127] Quantitative CSF yeast culture and its serial use for measurement of effective fungicidal activity has become a primary research tool for effectiveness of therapeutic regimens.[128] The quantitative yeast count has been correlated with outcome[129] and effective fungicidal activity has correlated with success of antifungal regimens, including survival.[95,128,130] Despite a decade of use and validation of its effectiveness in clinical studies, the use of quantitative CSF yeast culture for the determination of effective fungicidal activity has not yet become a part of routine clinical practice.

TREATMENT

Basic Principles

Amphotericin B deoxycholate (AmBd) is the cornerstone of treatment for severe cryptococcal infection, including meningoencephalitis. Treatment is summarized in Table 4. A standard induction dose of 0.7 to 1 mg/kg/d is recommended. Liposomal amphotericin B (3–6 mg/kg/d) has become a preferred alternative with similar outcomes and less nephrotoxicity, and is recommended specifically for primary induction in patients at risk for renal dysfunction.[93,131,132] Flucytosine (5-FC) is used in combination therapy with AmBd as first-line therapy in cryptococcal meningitis or severe pulmonary cryptococcosis at a dosage of 100 mg/kg/d in divided doses.[133,134] This combination represents the most potent fungicidal regimen, with faster CSF sterilization and fewer relapses, and is associated with lower attributable mortality.[133–139] Because the interruption of induction therapy is associated with poorer outcome, in resource-available areas the liposomal product has become the preferred polyene. Unfortunately, there are still no comparative studies with 5-FC combined with lipid formulations of amphotericin B as opposed to AmBd. Early mycological failure (defined as persistently positive CSF cultures at day 14) correlates with late treatment failure and poor outcome,[140] and lack of 5-FC is independently associated with both early[141] and late[137] mycological failure. This improved fungicidal activity of combination therapy translates into a direct survival benefit compared with AmBd monotherapy.[135] 5-FC should be dose adjusted for renal dysfunction, with therapeutic drug monitoring to decrease its primary side effect of bone marrow suppression.[142] There are emerging data that lower doses of 5-FC in combination with amphotericin may demonstrate similar fungicidal activity.[138]

Although combination induction therapy remains the recommended first-line therapy for severe cryptococcosis, 5-FC availability is limited in settings where the disease burden and mortality rates are the highest. Alternative combination therapies have been investigated, the most efficacious of which is AmBd plus fluconazole (800 mg/d), which results in improved rates of fungal clearance, neurologic recovery, and survival compared with AmBd alone or in combination with lower doses of fluconazole.[143,144] This combination offers a more feasible and potentially viable option for effective initial therapy in settings where access to 5-FC is limited. Optimizing treatment outcomes without exhausting limited resources is critical in many settings. Standardized fluid and electrolyte supplementation protocols for patients treated with amphotericin B in these resource-limited settings have been associated with improved early survival.[145] Additionally, shorter courses of amphotericin B in combination with other agents may be considered in these settings, although clinical endpoints for such regimens have not been rigorously evaluated.[146,147] An ongoing trial evaluating the combination of intermittent dosing of high-dose of liposomal amphotericin B with high-dose fluconazole in resource-limited settings is underway to address this unanswered question (AmBition-CM, www.controlled-trials.com/ ISRCTN10248064). Additional alternative induction regimens are available in the guidelines but their use is not encouraged based on limited data of the success with these regimens.[148] Fluconazole monotherapy for meningitis is not recommended for induction given its fungistatic nature, poor success, and higher relapse rates as well as increased rates of resistance in relapse.[93,94]

However, in areas without access to AmBd, high doses (≥1200 mg/d) of fluconazole should be commenced.

A 3-stage regimen of induction, consolidation, and maintenance is standard treatment for cryptococcal meningitis in all patients, irrespective of host risk factors.[93,133] In HIV-infected patients, initial induction treatment usually begins with combination therapy as described, followed by consolidation treatment with fluconazole (400–800 mg/d) for 8 weeks in patients who have demonstrated favorable response. Longer courses of both induction (eg, 6 weeks) and consolidation (or "eradication") therapy have been suggested in *C gattii* meningoencephalitis, irrespective of host immune status, owing to the observed severity of neurologic disease in this group of patients,[11,52,53] but this is not certain and in general *C gattii* should be treated similarly to *C neoformans*. After consolidation, long-term suppression is commenced with oral fluconazole (200–400 mg/d). This approach has decreased rates of relapse from approximately 40% to less than 5% in severely immunosuppressed patients.[149] Secondary prophylaxis is discontinued after 1 to 2 years of antifungal therapy in patients who respond to ART with an increase in $CD4^+$ cell counts to greater than 100 cells/μL and a decrease in HIV viral load to undetectable levels for at least 3 months.[93,150,151] The other triazoles (itraconazole, voriconazole, and posaconazole) are active against cryptococcal isolates in vitro and, in combination with AmBd, may have similar fungicidal activity to 5-FC,[144] but owing to differences in bioavailability, CSF penetration, drug interactions, cost, and lack of robust studies in cryptococcosis, these agents are not recommended as first-line agents for consolidation or maintenance therapy. However, they may have a role in refractory cases.[152–155]

Timing of Antiretroviral Therapy

In HIV-associated cryptococcal infection, ART has a major impact on long-term prognosis. However, several studies have suggested an increased risk of IRIS among HIV-infected patients initiated on ART early after the diagnosis of an opportunistic infection.[64,65,156] More contemporary studies have demonstrated conflicting results regarding outcomes of cryptococcal infection based on timing of ART initiation,[103,157,158] and studies in tuberculosis have demonstrated a survival benefit with earlier ART (despite increased rates of IRIS).[159,160] Recently, the Cryptococcal Optimal ART Timing Trial (COAT) provided some definitive guidance to delay initiation of ART in patients with cryptococcal meningitis for a minimum of 4 weeks after starting antifungal therapy. This randomized trial demonstrated improved survival in patients with cryptococcal meningitis in whom ART initiation was deferred for up to 5 weeks after diagnosis as compared with immediate ART (within 1–2 weeks).[161] Although increased rates of IRIS observed with early ART did not attain statistical significance, markers of macrophage activation were increased in this early group, suggesting that subclinical or compartmentalized IRIS may occur and influence mortality.[87,161]

Organ Transplant Recipients

Organ transplant recipients with CNS cryptococcal infection are managed similarly to HIV-infected patients, although lipid formulations of amphotericin B are preferred to limit nephrotoxicity.[93] A longer course of induction therapy is indicated if CSF cultures remain

positive at 2 weeks, because this scenario is associated with an increased 6-month mortality.[162] Relapse rates among organ transplant recipients are lower than in HIV-associated disease, such that a shorter course of maintenance therapy can be pursued following standard consolidation, but generally these patients are treated for 1 year.[93,162] Drug interactions between fluconazole and immunosuppressive agents should be anticipated owing to CYP3A4 inhibition, and a preemptive reduction in calcineurin inhibitors should be considered. Management of immunosuppression in the setting of cryptococcal infection requires recognition of the increased risk of IRIS.[77,80,163] Thus, stepwise reduction in immunosuppression is recommended, although the approach should be individualized for each patient.

Non–HIV-Infected, Nontransplant Patients

Very few prospective data are available on the management of cryptococcal infection in the apparently immunocompetent host lacking classical risk factors for cryptococcosis.[134] This heterogeneous group of patients is diagnosed later, irrespective of disease severity.[32,84] Recommendations for longer induction therapy (≥4 weeks) are based on the recognition of poorer outcomes and higher mortality rates in this group of patients both in early[134,164] as well as contemporary[32] studies. However, in patients with good prognostic factors and excellent antifungal induction response, 2-week induction therapy can be successful. Therapy should be extended further if 5-FC is not included (or there is limited exposure to this drug) in the induction regimen.[93] Recommendations for consolidation and maintenance parallel those for HIV-infected patients and reflect high relapse rates (30%) within the first year before the introduction of consolidation and maintenance antifungal strategies.[93,134] Criteria for stopping treatment in these patients include resolution of symptoms and at least 1 year of suppressive antifungal therapy.

Management of Intracranial Pressure

Along with the optimization of antifungal therapy, management of increased intracranial pressure is critically important in cryptococcal meningoencephalitis. Intracranial hypertension frequently corresponds with CSF fungal burden, potentially mediated by CSF outflow obstruction by clumped yeast forms even during early therapy, and is associated with increased morbidity and mortality.[97,165] Intracranial imaging should be performed before lumbar puncture if impaired mentation or focal neurologic deficits are present. A baseline CSF opening pressure should be obtained in all patients. Aggressive attempts to control increased intracranial pressure should occur when patients are symptomatic, although emerging data suggest there may be benefit to therapeutic lumbar punctures, irrespective of baseline opening pressure in resource-limited settings.[166] Treatment options for managing acutely elevated intracranial pressure include repeated lumbar punctures (daily until pressure and symptoms are stable for >2 days), lumbar drain insertion, ventriculostomy, or ventriculoperitoneal shunt, if obstructive hydrocephalus develops.[97] Consideration of early neurosurgical consultation has been recommended in cases of meningoencephalitis owing to *C gattii* where CNS inflammation is often severe.[52,53] Medical treatments such as corticosteroids (unless IRIS suspected or in cases of severe *C gattii* infection), mannitol, and acetazolamide are generally not recommended.[52,53,129,167] If

shunt placement is necessary, CSF sterilization is not required before insertion, which can be performed once appropriate antifungal therapy has been commenced.[168]

Persistent and Relapsed Infection

Persistent and relapsed infection must be distinguished from IRIS. Persistent disease has been defined as persistently positive CSF cultures after 1 month of antifungal therapy, whereas relapse requires new clinical signs and symptoms and positive cultures after initial improvement and fungal sterilization.[93] Surrogate markers, including biochemical parameters, India ink staining, and cryptococcal antigen titers, are insufficient to define relapse or alter antifungal therapy. General recommendations for management in these persistent or relapsed cases include resumption of induction therapy, often for a longer duration and at increased dosages, if tolerable, and pursuance of comparative antifungal susceptibility testing.[93] Although primary direct antifungal resistance to azoles and polyenes is rare, decreased susceptibility to fluconazole has been observed in some cases of culture-positive relapse.[94] There has not yet been a convincing minimum inhibitory concentration breakpoint for cryptococcal species in antifungal susceptibility testing; thus, the importance of comparative minimum inhibitory concentration testing with the original isolate in cases where resistance is suspected cannot be overemphasized.[169,170]

Nonmeningeal Disease

Although isolation of *Cryptococcus* from respiratory tract specimens can occur in the absence of clinical disease (colonization), it is incumbent upon the treating clinician to assess for subclinical disease or potential for complications when *Cryptococcus* is isolated from any clinical specimen. In the absence of immune compromise, airway colonization carries a low risk for invasive disease and treatment can be deferred; although in most cases, given the safety profile of fluconazole, many clinicians favor treatment in all patients in whom *Cryptococcus* is isolated. In immunosuppressed patients with isolated pulmonary cryptococcosis, however, treatment is recommended to prevent dissemination.[93] This group of patients should be evaluated for systemic disease (including blood and CSF cultures as well as CrAg testing from serum and CSF) to optimize treatment. In any patient in whom cryptococcemia is identified, symptoms are severe, or CSF examination reveals asymptomatic CNS involvement, treatment for cryptococcal meningitis is recommended.[93] The potential for severe pulmonary infection owing to *C gattii* should be appreciated when *Cryptococcus* is isolated from respiratory cultures in settings where this species is endemic[11,12,52,53,171]; however, to date, there are no convincing data that species identification is required to optimally select antifungal therapy, and disease severity remains the critical factor in determining initial treatment. Cerebral cryptococcomas often can be managed with prolonged antifungal therapy without the need for surgical removal unless mass effect or other evidence of obstruction is identified. A longer induction phase with AmBd plus 5-FC, followed by 6 to 18 months of consolidation therapy with fluconazole (400–800 mg/d) is recommended. Localized infection of extrapulmonary nonmeningeal sites can occur occasionally with direct inoculation, but more commonly represents disseminated infection. Suspicion for the latter must be maintained when *Cryptococcus* is identified from a sterile body site, because management strategies differ if disseminated disease is present. Consultation with ophthalmology is indicated in cases of cryptococcal eye disease.[93]

Screening and Prevention

There is no question that early identification of HIV infection and initiation of ART in patients before progression to severe immunodeficiency is the most effective intervention at reducing the global burden of cryptococcosis and other opportunistic infections. However, despite increased access to ART worldwide, late presentations of HIV infection still occur and the burden of severe cryptococcal infection and related mortality remains disproportionately represented in these populations.

Fluconazole prophylaxis has been shown to be effective for preventing cryptococcosis in patients with advanced AIDS in endemic areas[172,173]; however, universal prophylaxis is relatively cost ineffective,[124] has not been shown to offer a survival benefit,[174] and may add to the appearance of azole-resistant strains. As such, this approach is not recommended currently.

Given that mortality from cryptococcal meningitis remains unacceptably high, alternative management strategies have been evaluated and implemented in resource-limited settings, specifically a “screen and treat” approach using serum cryptococcal antigen (CrAg) testing followed by preemptive fluconazole therapy in CrAg-positive patients. CrAg is an early marker of cryptococcal disease, detectable in serum a median of 22 days before the onset of symptoms, and is both highly predictive of incident cryptococcal meningitis and an independent risk factor for death during the first year of ART.[175–177] This approach is associated with a decreased incidence of cryptococcal meningitis and improved survival among patients with advanced HIV disease and has been successfully implemented in several resource-limited settings, with a baseline prevalence of asymptomatic cryptococcal antigenemia of 5% to 13%.[177,178] Moreover, analyses have consistently demonstrated both the cost effectiveness and survival advantage of a “screen and treat” approach, as compared with standard of care or universal fluconazole prophylaxis, at CrAg prevalences as low as 0.6%.[178–180] As access to lateral flow assay testing in these settings is increased, the cost effectiveness is likely to be greater than initially reported. The World Health Organization now recommends implementation of CrAg screening and preemptive fluconazole therapy in ART-naïve adults with a CD4 count of less than 100 cells/mm^3 before initiating ART in endemic settings.[181] Several nations in sub-Saharan Africa have since operationalized programs as a part of the existing HIV infrastructure. Several unanswered questions remain, however, including the feasibility of implementation, the dose and duration of preemptive fluconazole, the criteria for lumbar puncture in asymptomatic patients, and the potential impact on azole resistance. Some data suggest a ‘screen and treat’ would be cost effective, even in resource-rich settings, although this is currently not part of standard practice, despite recent reports of CrAg prevalence of more than 3% in the United States.[176,182] Routine screening for cryptococcal infection and/or prophylaxis are not recommended in solid organ transplant recipients, even when immunosuppression is augmented in patients with previously (appropriately) treated infection.[183]

In the arena of direct immune modulation for cryptococcosis management, aside from the use of ART, progress has been slow. First, although both cryptococcal glucuronoxylomannan–tetanus toxoid conjugate vaccine and specific monoclonal antibodies to cryptococci have been developed, clinical trials have not been initiated to determine their

usefulness in human subjects.[184,185] The use of immune stimulation with recombinant gamma-interferon has both immunologic support and 2 positive clinical trials,[186–189] but has only been used in refractory cases and likely reflects concerns about precisely judging immune stimulation when IRIS can be a deadly problem.

References

1. Knoke M, Schwesinger G. One hundred years ago: the history of cryptococcosis in Greifswald. Medical mycology in the nineteenth century. Mycoses. 1994; 37:229–33. [PubMed: 7739651]
2. Casadevall, A., Perfect, JR. Cryptococcus neoformans. Washington, DC: ASM Press; 1998.
3. Park BJ, Wannemuehler KA, Marston BJ, et al. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS. 2009; 23:525–30. [PubMed: 19182676]
4. 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 U S A. 2004; 101:17258–63. [PubMed: 15572442]
5. MacDougall L, Fyfe M, Romney M, et al. Risk factors for *Cryptococcus gattii* infection, British Columbia, Canada. Emerg Infect Dis. 2011; 17(2):193–9. [PubMed: 21291588]
6. Marr K, Datta K, Pirofski L, et al. *Cryptococcus gattii* Infection in healthy Hosts: A Sentinel for Subclinical Immunodeficiency? Clin Infect Dis. 2012; 54(1):153–4. [PubMed: 22075791]
7. Rosen L, Freeman A, Yang L, et al. Anti-GM-CSF autoantibodies in patients with cryptococcal meningitis. J Immunol. 2013; 190:3959–66. [PubMed: 23509356]
8. Saijo T, Chen J, Chen S, et al. Anti-granulocyte macrophage colony-stimulating factor autoantibodies are a risk factor for central nervous system infection by *Cryptococcus gattii* in otherwise immunocompetent patients. MBio. 2014; 5(2):e00912–4. [PubMed: 24643864]
9. Mitchell D, Sorrell T, Allworth A, 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. [PubMed: 7756484]
10. Harris J, Lockhart S, Debess E, et al. *Cryptococcus gattii* in the United States: clinical aspects of infection with an emerging pathogen. Clin Infect Dis. 2011; 53:1188–95. [PubMed: 22016503]
11. Chen S, Meyer W, Sorrell T. *Cryptococcus gattii* infections. Clin Microbiol Rev. 2014; 27(4):980–1024. [PubMed: 25278580]
12. Phillips P, Galanis E, MacDougall L, et al. Longitudinal clinical findings and outcome among patients with *Cryptococcus gattii* infection in British Columbia. Clin Infect Dis. 2015; 60(9):1368–76. [PubMed: 25632012]
13. Hagen F, Khayhan K, Theelen B, et al. Recognition of seven species in the *Cryptococcus gatti/Cryptococcus neoformans* species complex. Fungal Genet Biol. 2015; 78:16–48. [PubMed: 25721988]
14. Chen S, Sorrell T, Nimmo G, et al. Epidemiology and host- and variety-dependent characteristics of infection due to *Cryptococcus neoformans* in Australia and New Zealand. Australasian Cryptococcal Study Group. Clin Infect Dis. 2000; 31:499–508. [PubMed: 10987712]
15. Chen Y, Litvintseva A, Frazzitta A, et al. Comparative analyses of clinical and environmental populations of Cryptococcus neoformans in Botswana. Mol Ecol. 2015; 24(14):3559–71. [PubMed: 26053414]
16. Emmons C. Saprophytic sources of Cryptococcus neoformans associated with the pigeon (*Columba livia*). Am J Hyg. 1955; 62(3):227–32. [PubMed: 13268414]
17. Steenbergen J, Shuman H, Casadevall A. *Cryptococcus neoformans* interactions with amoebae suggest an explanation for its virulence and intracellular pathogenic strategy in macrophages. Proc Natl Acad Sci U S A. 2001; 98:15245. [PubMed: 11742090]
18. Ruiz A, Neilson J, Bulmer G. Control of *Cryptococcus neoformans* in nature by biotic factors. Sabouraudia. 1982; 20(1):21–9. [PubMed: 6801787]
19. Ellis D, Pfeiffer T. Natural habitat of *Cryptococcus neoformans* var gattii. J Clin Microbiol. 1990; 28:1642–4. [PubMed: 2199524]

20. Hoang L, Maguire J, Doyle P, et al. Cryptococcus neoformans infections at Vancouver Hospital and Health Sciences Centre (1997–2002): epidemiology, microbiology and histopathology. J Med Microbiol. 2004; 53(Pt 9):935. [PubMed: 15314203]
21. Datta K, Bartlett K, Baer R, et al. Spread of *Cryptococcus gattii* into Pacific Northwest region of the United States. Emerg Infect Dis. 2009; 15(8):1185–91. [PubMed: 19757550]
22. Springer D, Chaturvedi V. Projecting global occurrence of *Cryptococcus gattii*. Emerg Infect Dis. 2010; 16(1):14–20. [PubMed: 20031037]
23. Hull CM, Heitman J. Genetics of *Cryptococcus neoformans*. Annu Rev Genet. 2002; 36:557–615. [PubMed: 12429703]
24. Lin X, Hull C, Heitman J. Sexual reproduction between partners of the same mating type in Cryptococcus neoformans. Nature. 2005; 434:1017–21. [PubMed: 15846346]
25. Sukroongreung S, Kitiniyom K, Nilakul X, et al. Pathogenicity of basidiospores of *Filobasidiella neoformans* var. neoformans. Med Mycol. 1998; 36(6):419–24. [PubMed: 10206753]
26. Fraser J, Giles S, Wenink E, et al. Same-sex mating and the origin of the Vancouver Island *Cryptococcus gattii* outbreak. Nature. 2005; 437:1360–4. [PubMed: 16222245]
27. Byrnes E, Li W, Lewit Y, et al. Emergence and pathogenicity of highly virulent *Cryptococcus gattii* genotypes in the Northwest United States. PLoS Pathog. 2010; 6(4):e1000850. [PubMed: 20421942]
28. Litvintseva A, Marra R, Nielsen K, et al. Evidence of sexual recombination among *Cryptococcus neoformans* serotype A isolates in sub-Saharan Africa. Eukaryot Cell. 2003; 2(6):1162–8. [PubMed: 14665451]
29. Hajjeh RA, Conn LA, Stephens DS, et al. Cryptococcosis: population-based multistate active surveillance and risk factors in human immunodeficiency virus-infected persons. Cryptococcal Active Surveillance Group. J Infect Dis. 1999; 179:449–54. [PubMed: 9878030]
30. Perfect JR, Casadevall A. Cryptococcosis. Infect Dis Clin North Am. 2002; 16:837–74. [PubMed: 12512184]
31. Perfect, JR. Cryptococcus neoformans and *Cryptococcus gattii*. In: Bennett, JE.Dolin, R., Blaser, MJ., editors. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. 8th. Philadelphia: Elsevier Saunders; 2015. p. 2934-48.
32. Bratton E, El Husseini N, Chastain C, et al. Comparison and temporal trends of three groups with Cryptococcosis: HIV-infected, solid organ transplant, and HIV-negative/non-transplant. PLoS One. 2012; 7(8):e43582. [PubMed: 22937064]
33. Christianson J, Engber W, Andes D. Primary cutaneous cryptococcosis in immunocompetent and immunocompromised hosts. Med Mycol. 2003; 41(3):177–88. [PubMed: 12964709]
34. Goldman D, Khine H, Abadi J, et al. Pediatrics. 2001; 107(5):E66. [PubMed: 11331716]
35. Shao X, Mednick A, Alvarez M, et al. An innate immune system cell is a major determinant in species-related susceptibility to fungal pneumonia. J Immunol. 2005; 175:3244–51. [PubMed: 16116215]
36. Perfect JR. *Cryptococcus neoformans*: a sugar-coated killer with designer genes. FEMS Immunol Med Microbiol. 2005; 45:395–404. [PubMed: 16055314]
37. Sun H, Alexander B, Lortholary O, et al. Unrecognized pretransplant and donor-derived cryptococcal disease in organ transplant recipients. Clin Infect Dis. 2010; 51(9):1062–9. [PubMed: 20879857]
38. Krockenberger M, Malik R, Ngamskulrungroj P, et al. Pathogenesis of pulmonary *Cryptococcus gattii* infection: a rat model. Mycopathologia. 2010; 170(5):315–30. [PubMed: 20552280]
39. Santangelo R, Zoellner H, Sorrell T, et al. Role of extracellular phospholipases and mononuclear phagocytes in dissemination of cryptococcosis in a murine model. Infect Immun. 2004; 72:1693–9. [PubMed: 14977977]
40. Maruvada R, Zhu L, Pearce D, et al. Cryptococcus neoformans phospholipase B1 activates host cell Rac1 for traversal across the blood-brain barrier. Cell Microbiol. 2012; 14(10):1544–53. [PubMed: 22646320]
41. Ahi M, Li S, Zheng C, et al. Real-time imaging of trapping and urease-dependent transmigration of *Cryptococcus neoformans* in mouse brain. J Clin Invest. 2010; 120(5):1683–93. [PubMed: 20424328]

42. Charlier C, Nielsen K, Daou S, et al. Evidence of a role for monocytes in dissemination and brain invasion by Cryptococcus neoformans. Infect Immun. 2009; 77(1):120–7. [PubMed: 18936186]
43. Casadevall A. Cryptococci at the brain gate: break and enter or use a Trojan horse? J Clin Invest. 2010; 120(5):1389–92. [PubMed: 20424319]
44. Coelho C, Bocca A, Casadevall A. The tools for virulence of Cryptococcus neoformans. Adv Appl Microbiol. 2014; 87:1–41. [PubMed: 24581388]
45. Okagaki L, Strain A, Nielsen J, et al. Cryptococcal cell morphology affects host cell interactions and pathogenicity. PLoS Pathog. 2010; 6:e1000953. [PubMed: 20585559]
46. Zaragoza O, Garcia-Rodas R, Nosanchuk J, et al. Fungal cell gigantism during mammalian infection. PLoS Pathog. 2010; 6:e1000945. [PubMed: 20585557]
47. Tucker S, Casadevall A. Replication of *Cryptococcus neoformans* in macrophages is accompanied by phagosomal permeabilization and accumulation of vesicles containing polysaccharide in the cytoplasm. Proc Natl Acad Sci U S A. 2002; 99(5):3165–70. [PubMed: 11880650]
48. Alvarez M, Casadevall A. Phagosome extrusion and host-cell survival after *Cryptococcus neoformans* phagocytosis by macrophages. Curr Biol. 2006; 16:2161–5. [PubMed: 17084702]
49. Geunes-Boyer S, Beers M, Perfect J, et al. Surfactant protein D facilitates Cryptococcus neoformans infection. Infect Immun. 2012; 80(7):2444–53. [PubMed: 22547543]
50. Warr W, Bates JH, Stone A. The spectrum of pulmonary cryptococcosis. Ann Intern Med. 1968; 69:1109–16. [PubMed: 5725729]
51. Brizendine K, Baddley J, Pappas P. Pulmonary cryptococcosis. Semin Respir Crit Care Med. 2011; 32(6):727–34. [PubMed: 22167400]
52. Chen S, Korman T, Slavin M, et al. Antifungal therapy and management of complications of cryptococcosis due to *Cryptococcus Gattii*. Clin Infect Dis. 2013; 57(4):543–51. [PubMed: 23697747]
53. Franco-Paredes C, Womack T, Bohlmeyer T, et al. Management of *Cryptococcus gattii* meningoencephalitis. Lancet Infect Dis. 2015; 15:348–55. [PubMed: 25467646]
54. Speed B, Dunt D. Clinical and host differences between infections with the two varieties of Cryptococcus neoformans. Clin Infect Dis. 1995; 21:28–34. [PubMed: 7578756]
55. Singh N, Gayowski T, Wagener MM, et al. Clinical spectrum of invasive cryptococcosis in liver transplant recipients receiving tacrolimus. Clin Transplant. 1997; 11:66–70. [PubMed: 9067698]
56. Odom A, Muir S, Lim E, et al. Calcineurin is required for virulence of Crytptococcus neoformans. EMBO J. 2007; 16:2576–89.
57. Allen R, Barter CE, Cachou LL, et al. Disseminated cryptococcosis after transurethral resection of the prostate. Aust N Z J Med. 1982; 12:296–9. [PubMed: 6958242]
58. Larsen RA, Bozzette S, McCutchan JA, et al. Persistent Cryptococcus neoformans infection of the prostate after successful treatment of meningitis. California Collaborative Treatment Group. Ann Intern Med. 1989; 111:125–8. [PubMed: 2545124]
59. Staib F, Seibold M, L'Age M. Persistence of *Cryptococcus neoformans* in seminal fluid and urine under itraconazole treatment. The urogenital tract (prostate) as a niche for *Cryptococcus neoformans*. Mycoses. 1990; 33:369–73. [PubMed: 1965324]
60. Okun E, Butler WT. Ophthalmologic complications of cryptococcal meningitis. Arch Ophthalmol. 1964; 71:52–7. [PubMed: 14066039]
61. Rex JH, Larsen RA, Dismukes WE, et al. Catastrophic visual loss due to *Cryptococcus neoformans* meningitis. Medicine (Baltimore). 1993; 72:207–24. [PubMed: 8341139]
62. Liu PY. Cryptococcal osteomyelitis: case report and review. Diagn Microbiol Infect Dis. 1998; 30(1):33–5. [PubMed: 9488829]
63. Albert-Braun S, Venema F, Bausch J, et al. *Cryptococcus neoformans* peritonitis in a patient with alcoholic cirrhosis: case report and review of the literature. Infection. 2005; 33:282–8. [PubMed: 16091901]
64. Shelburne SA, Visnegarwala F, Darcourt J, et al. Incidence and risk factors for immune reconstitution inflammatory syndrome during HAART. AIDS. 2005; 19(4):399–406. [PubMed: 15750393]

65. Lortholary O, Fontanet A, Memain N, et al. Incidence and risk factors of immune reconstitution inflammatory syndrome complicating HIV-associated cryptococcosis in France. AIDS. 2005; 19(10):1043–9. [PubMed: 15958835]
66. 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(2):130–4. [PubMed: 19365271]
67. Sungkanuparph S, Filler SG, Chetchotisakd P, et al. Cryptococcal immune reconstitution inflammatory syndrome after HAART in AIDS patients with cryptococcal meningitis: a prospective multicenter study. Clin Infect Dis. 2009; 49(6):931–4. [PubMed: 19681708]
68. Boulware DR, Meya DB, Bergemann TL, et al. Clinical features and serum biomarkers in HIV immune reconstitution inflammatory syndrome after cryptococcal meningitis: a prospective cohort study. PLoS Med. 2010; 7(12):e1000384. [PubMed: 21253011]
69. Haddow LJ, Easterbrook PJ, Mosam A, et al. Defining immune reconstitution inflammatory syndrome: evaluation of expert opinion versus 2 case definitions in a South African cohort. Clin Infect Dis. 2009; 49(9):1424–32. [PubMed: 19788360]
70. Shelburne SA 3rd, Darcourt J, White AC Jr, et al. The role of immune reconstitution inflammatory syndrome in AIDS-related *Cryptococcus neoformans* disease in the era of HAART. Clin Infect Dis. 2005; 40(7):1049–52. [PubMed: 15825000]
71. Sungkanuparph S, Jongwutiwes U, Kiertiburanakul S. Timing of cryptococcal immune reconstitution inflammatory syndrome after HAART in patients with AIDS and cryptococcal meningitis. J Acquir Immune Defic Syndr. 2007; 45(5):595–6. [PubMed: 17704683]
72. Kambugu A, Meya DB, Rhein J, et al. Outcomes of cryptococcal meningitis in Uganda before and after the availability of HAART. Clin Infect Dis. 2008; 46(11):1694–701. [PubMed: 18433339]
73. Boulware DR, Bonham SC, Meya DB, et al. Paucity of initial cerebrospinal fluid inflammation in cryptococcal meningitis is associated with subsequent immune reconstitution inflammatory syndrome. J Infect Dis. 2010; 202(6):962–70. [PubMed: 20677939]
74. da Cunha Colombo ER, Mora DJ, Silva-Vergara ML. Immune reconstitution inflammatory syndrome (IRIS) associated with *Cryptococcus neoformans* infection in AIDS patients. Mycoses. 2011; 54(4):e178–82. [PubMed: 20337940]
75. Rambeloarisoa J, Batisse D, Thiebaut JB, et al. Intramedullary abscess resulting from disseminated cryptococcosis despite immune restoration in a patient with AIDS. J Infect. 2002; 44(3):185–8. [PubMed: 12099747]
76. Skiest DJ, Hester LJ, Hardy RD. Cryptococcal immune reconstitution inflammatory syndrome: report of four cases in three patients and review of the literature. J Infect. 2005; 51(5):e289–97. [PubMed: 16321643]
77. Singh N, Lortholary O, Alexander BD, et al. Allograft loss in renal transplant recipients with *Cryptococcus neoformans* associated immune reconstitution syndrome. Transplantation. 2005; 80(8):1131–3. [PubMed: 16278598]
78. Conti HR, Shen F, Nayyar N, et al. Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against oral candidiasis. J Exp Med. 2009; 206(2):299–311. [PubMed: 19204111]
79. Sun HY, Singh N. Opportunistic infection-associated immune reconstitution syndrome in transplant recipients. Clin Infect Dis. 2011; 53(2):168–76. [PubMed: 21690625]
80. Lanternier F, Chandesris MO, Poiree S, et al. Cellulitis revealing a cryptococcosis-related immune reconstitution inflammatory syndrome in a renal allograft recipient. Am J Transplant. 2007; 7(12): 2826–8. [PubMed: 17927804]
81. Crespo G, Cervera C, Michelena J, et al. Immune reconstitution syndrome after voriconazole treatment for cryptococcal meningitis in a liver transplant recipient. Liver Transplant. 2008; 14(11):1671–4.
82. Singh N. Novel immune regulatory pathways and their role in immune reconstitution syndrome in organ transplant recipients with invasive mycoses. Eur J Clin Microbiol Infect Dis. 2008; 27(6): 403–8. [PubMed: 18214557]

83. Sun H, Alexander B, Huprikar S, et al. Predictors of immune reconstitution syndrome in organ transplant recipients with cryptococcosis: implications for the management of immunosuppression. Clin Infect Dis. 2015; 60(1):36–44. [PubMed: 25210020]
84. Ecevit IZ, Clancy CJ, Scmalfuss IM, et al. The poor prognosis of central nervous system cryptococcosis among nonimmunosuppressed patients: a call for better disease recognition and evaluation of adjuncts to antifungal therapy. Clin Infect Dis. 2006; 42:1443–7. [PubMed: 16619158]
85. Haddow LJ, Colebunders R, Meintjes G, et al. Cryptococcal immune reconstitution inflammatory syndrome in HIV-1-infected individuals: proposed clinical case definitions. Lancet Infect Dis. 2010; 10(11):791–802. [PubMed: 21029993]
86. Tan DB, Yong YK, Tan HY, et al. Immunological profiles of immune restoration disease presenting as mycobacterial lymphadenitis and cryptococcal meningitis. HIV Med. 2008; 9(5):307–16. [PubMed: 18400078]
87. Scriven J, Rhein J, Hullsiek K, et al. Early ART after cryptococcal meningitis is associated with cerebrospinal fluid pleocytosis and macrophage activation in a multisite randomized trial. J Infect Dis. 2015; 212(5):769–78. [PubMed: 25651842]
88. Jarvis J, Meintjes G, Bicanic T, et al. Cerebrospinal fluid cytokine profiles predict risk of early mortality and immune reconstitution inflammatory syndrome in HIV-associated cryptococcal meningitis. PLoS Pathog. 2014; 11(4):e1004754.
89. Chang C, Omarjee S, Lim A, et al. Chemokine levels and chemokine receptor expression in the blood and the cerebrospinal fluid of HIV-infected patients with cryptococcal meningitis and cryptococcosis-associated immune reconstitution syndrome. J Infect Dis. 2013; 208:1604–12. [PubMed: 23908492]
90. Trevenzoli M, Cattelan AM, Rea F, et al. Mediastinitis due to cryptococcal infection: a new clinical entity in the HAART era. J Infect. 2002; 45(3):173–9. [PubMed: 12387774]
91. Blanche P, Gombert B, Ginsburg C, et al. HIV combination therapy: immune restitution causing cryptococcal lymphadenitis dramatically improved by anti-inflammatory therapy. Scand J Infect Dis. 1998; 30(6):615–6. [PubMed: 10225395]
92. Meintjes G, Lawn SD, Scano F, et al. Tuberculosis-associated immune reconstitution inflammatory syndrome: case definitions for use in resource-limited settings. Lancet Infect Dis. 2008; 8(8):516–23. [PubMed: 18652998]
93. Perfect JR, Dismukes WE, Dromer F, et al. Clinical Practice Guide lines for the Management of Cryptococcal Disease: 2010 update by the Infectious Disease Society of America. Clin Infect Dis. 2010; 50:291–322. [PubMed: 20047480]
94. Bicanic T, Harrison T, Niepieklo A, et al. Symptomatic relapse of HIV-associated cryptococcal meningitis after initial fluconazole monotherapy: the role of fluconazole resistance and immune reconstitution. Clin Infect Dis. 2006; 43(8):1069–73. [PubMed: 16983622]
95. Bicanic T, Muzoora C, Brouwer AE, et al. Independent association between rate of clearance of infection and clinical outcome of HIV-associated cryptococcal meningitis: analysis of a combined cohort of 262patients. Clin Infect Dis. 2009; 49(5):702–9. [PubMed: 19613840]
96. Meintjes G, Wilkinson RJ, Morroni C, et al. Randomized placebo-controlled trial of prednisone for paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome. AIDS. 2010; 24(15):2381–90. [PubMed: 20808204]
97. Graybill JR, Sobel J, Saag M, et al. Diagnosis and management of increased intracranial pressure in patients with AIDS and cryptococcal meningitis. The NIAID Mycoses Study Group and AIDS Cooperative Treatment Groups. Clin Infect Dis. 2000; 30(1):47–54. [PubMed: 10619732]
98. Narayanan S, Banerjee C, Holt PA. Cryptococcal immune reconstitution syndrome during steroid withdrawal treated with hydroxychloroquine. Int J Infect Dis. 2011; 15(1):e70–3. [PubMed: 21094071]
99. Sitapati AM, Kao CL, Cachay ER, et al. Treatment of HIV-related inflammatory cerebral cryptococcoma with adalimumab. Clin Infect Dis. 2010; 50(2):e7–10. [PubMed: 20001539]
100. Scemla A, Gerber S, Duquesne A, et al. Dramatic improvement of severe cryptococcosis-induced immune reconstitution syndrome with adalimumab in a renal transplant recipient. Am J Transplant. 2015; 15:560–4. [PubMed: 25611999]

101. Brunel A, Reynes J, Tuaillon E, et al. Thalidomide for steroid-dependent immune reconstitution inflammatory syndromes during AIDS. AIDS. 2012; 26(16):2110–2. [PubMed: 22874513]
102. Biagetti C, Nicola M, Borderi M, et al. Paradoxical immune reconstitution inflammatory syndrome associated with previous *Cryptococcus neoformans* infection in an HIV-positive patient requiring neurosurgical intervention. New Microbiol. 2009; 32(2):209–12. [PubMed: 19579702]
103. Zolopa A, Andersen J, Powderly W, et al. Early HAART reduces AIDS progression/death in individuals with acute opportunistic infections: a multicenter randomized strategy trial. PLoS One. 2009; 4(5):e5575. [PubMed: 19440326]
104. French MA. HIV/AIDS: immune reconstitution inflammatory syndrome: a reappraisal. Clin Infect Dis. 2009; 48(1):101–7. [PubMed: 19025493]
105. Diamond RD, Bennett JE. Prognostic factors in cryptococcal meningitis. A study in 111 cases. Ann Intern Med. 1974; 80:176–81. [PubMed: 4811791]
106. Antinori S. New Insights into HIV/AIDS-Associated Cryptococcosis. ISRN AIDS. 2013; 2013:471363. [PubMed: 24052889]
107. Shibuya K, Coulson WF, Wollman JS, et al. Histopathology of cryptococcosis and other fungal infections in patients with acquired immunodeficiency syndrome. Int J Infect Dis. 2001; 5:78–85. [PubMed: 11468102]
108. Sato Y, Osabe S, Kuno H, et al. Rapid diagnosis of cryptococcal meningitis by microscopic examination of centrifuged cerebrospinal fluid sediment. J Neurol Sci. 1999; 164:72–5. [PubMed: 10385051]
109. Kanjanavirojkul N, Sripa C, Puapairoj A. Cytologic diagnosis of *Cryptococcus neoformans* in HIV-positive patients. Acta Cytol. 1997; 41:493–6. [PubMed: 9100786]
110. Malabonga VM, Basti J, Kamholz SL. Utility of bronchoscopic sampling techniques for cryptococcal disease in AIDS. Chest. 1991; 99:370–2. [PubMed: 1989797]
111. Lee LN, Yang PC, Kuo SH, et al. Diagnosis of pulmonary cryptococcosis by ultrasound guided percutaneous aspiration. Thorax. 1993; 48:75–8. [PubMed: 8434359]
112. Tanner DC, Weinstein MP, Fedorciw B, et al. Comparison of commercial kits for detection of cryptococcal antigen. J Clin Microbiol. 1994; 32:1680–4. [PubMed: 7929757]
113. Wu TC, Koo SY. Comparison of three commercial cryptococcal latex kits for detection of cryptococcal antigen. J Clin Microbiol. 1983; 18:1127–30. [PubMed: 6643665]
114. Stamm AM, Polt SS. False-negative cryptococcal antigen test. JAMA. 1980; 244:1359. [PubMed: 6997519]
115. Bloomfield N, Gordon MA, Elmendorf DF. Detection of *Cryptococcus neoformans* antigen in body fluids by latex particle agglutination. Proc Soc Exp Biol Med. 1963; 114:64–7. [PubMed: 14076914]
116. Jarvis JN, Percival A, Bauman S, et al. Evaluation of a novel point-of-care cryptococcal antigen test on serum, plasma and urine from patients with HIV-associated cryptococcal meningitis. Clin Infect Dis. 2011; 53:1019–23. [PubMed: 21940419]
117. Lindsley MD, Mekha N, Baggett HC, et al. Evaluation of a newly developed lateral flow immunoassay for the diagnosis of Cryptococcus. Clin Infect Dis. 2011; 53:321–5. [PubMed: 21810743]
118. McMullan BJ, Halliday C, Sorrell TC, et al. Clinical utility of the cryptococcal antigen lateral flow assay in a diagnostic mycology laboratory. PLoS One. 2012; 7:e49451. [PubMed: 23185334]
119. Binnicker MJ, Jespersen DJ, Bestrom JE, et al. Comparison of four assays for the detection of cryptococcal antigen. Clin Vaccine Immunol. 2012; 19:1988–90. [PubMed: 23081814]
120. Hansen J, Slechta ES, Gates-Hollingsworth MA, et al. Large-scale evaluation of the Immuno-Mycologics lateral flow and Enzyme-Linked immunoassays for detection of cryptococcal antigen in serum and cerebrospinal fluid. Clin Vaccine Immunol. 2013; 20:52–5. [PubMed: 23114703]
121. Huang H, Fan L, Rajbanshi B, et al. Evaluation of a new cryptococcal antigen lateral flow immunoassay in serum, cerebrospinal fluid and urine for the diagnosis of cryptococcosis: a meta-analysis and systematic review. PLoS One. 2015; 10(5):e0127117. [PubMed: 25974018]

122. Kabanda T, Siedner M, Klausner J, et al. Point-of-care diagnosis and prognostication of cryptococcal meningitis with the cryptococcal lateral flow assay on cerebrospinal fluid. Clin Infect Dis. 2014; 58(1):113–6. [PubMed: 24065327]

123. Williams D, Kiiza T, Kwizera R, et al. Evaluation of fingerstick cryptococcal antigen lateral flow assay in HIV-infected persons: a diagnostic accuracy study. Clin Infect Dis. 2015; 61(3):464–7. [PubMed: 25838287]

124. Jarvis JN, Harrison TS, Lawn SD, et al. Cost effectiveness of cryptococcal antigen screening as a strategy to prevent cryptococcal meningitis in South Africa. PLoS One. 2013; 8:e69288. [PubMed: 23894442]

125. Bindschadler DD, Bennett JE. Serology of human cryptococcosis. Ann Intern Med. 1968; 69:45–52. [PubMed: 4872700]

126. Saag MS, Powderly WG, Cloud GA, et al. Comparison of amphotericin B with fluconazole in the treatment of acute AIDS-associated cryptococcal meningitis. The NIAID Mycoses Study Group and the AIDS Clinical Trials Group. N Engl J Med. 1992; 326(8):3–89.

127. Powderly WG, Cloud GA, Dismukes WE, et al. 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. [PubMed: 8075272]

128. 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(1):76–80. [PubMed: 17554704]

129. Jarvis J, Bicanic T, Loyse A, et al. Determinants of mortality in a combined cohort of 501 patients With HIV-associated cryptococcal meningitis: implications for improving outcomes. Clin Infect Dis. 2014; 58(5):736–45. [PubMed: 24319084]

130. Perfect J, Bicanic T. Cryptococcosis diagnosis and treatment: what do we know now. Fungal Genet Biol. 2015; 78:49–54. [PubMed: 25312862]

131. 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. 1997; 11:1463–71. [PubMed: 9342068]

132. Hamill RJ, Sobel JD, El-Sadr W, et al. Comparison of 2 doses of liposomal amphotericin B and conventional amphotericin B deoxycholate for treatment of AIDS-associated acute cryptococcal meningitis: a randomized, double-blind clinical trial of efficacy and safety. Clin Infect Dis. 2010; 51(2):225–32. [PubMed: 20536366]

133. van der Horst CM, Saag MS, Cloud GA, et al. Treatment of cryptococcal meningitis associated with the acquired immunodeficiency syndrome. National Institute of Allergy and Infectious Diseases Mycoses Study Group and AIDS Clinical Trials Group. N Engl J Med. 1997; 337:15–21. [PubMed: 9203426]

134. 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. [PubMed: 3299095]

135. Day JN, Chau T, Wolbers M, et al. Combination antifungal therapy for cryptococcal meningitis. N Engl J Med. 2013; 368:1291–302. [PubMed: 23550668]

136. Brouwer AE, Rajanuwong A, Chieraku W, et al. Combination antifungal therapies for HIV-associated cryptococcal meningitis: a randomised trial. Lancet. 2004; 363:1764–7. [PubMed: 15172774]

137. Dromer F, Bernede-Bauduin C, Guillemot D, et al. Major role for amphotericin-flucytosine combination in severe cryptococcosis. PLoS One. 2008; 3(8):e2870. [PubMed: 18682846]

138. O'Connor L, Livermore J, Sharp A, et al. Pharmacodynamics of liposomal amphotericin B and flucytosine for cryptococcal meningoencephalitis: safe and effective regimens for immunocompromised patients. J Infect Dis. 2013; 208:351–61. [PubMed: 23599314]

139. Bratton E, El Husseini N, Chastain C, et al. Approaches to antifungal therapies and their effectiveness among patients with cryptococcosis. Antimicrob Agents Chemother. 2013; 57(6): 2485–95. [PubMed: 23478968]

140. Robinson PA, Bauer M, Leal MAE, et al. Early mycological treatment failure in AIDS-associated cryptococcal meningitis. Clin Infect Dis. 1999; 28:82–92. [PubMed: 10028076]

141. Dromer F, Mathoulin-Pelissier S, Launay O, et al. Determinants of disease presentation and outcome during cryptococcosis: The Crypto A/D Study. PLoS Med. 2007; 4:e21. [PubMed: 17284154]
142. Drew, RH., Perfect, JR. Flucytosine. In: Yu, V.Weber, R., Raoult, D., editors. Antimicrobial therapy and vaccines. New York: Apple Trees Productions; 1997. p. 656-7.
143. 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. [PubMed: 19441980]
144. Loyse A, Wilson D, Meintjes G, et al. Comparison of the early fungicidal activity of high-dose fluconazole, voriconazole, and flucytosine as second-line drugs given in combination with amphotericin B for the treatment of HIV-associated cryptococcal meningitis. Clin Infect Dis. 2012; 54:121–8. [PubMed: 22052885]
145. Bahr N, Rolfes M, Musubire A, et al. Standardized electrolyte supplementation and fluid management improves survival during amphotericin therapy for cryptococcal meningitis in resource-limited settings. Open Forum Infect Dis. 2014; 1(2):ofu070. [PubMed: 25734140]
146. Muzoora C, Kabanda T, Ortu G, et al. Short course amphotericin B with high dose fluconazole for HIV-associated cryptococcal meningitis. J Infect. 2012; 64:76–81. [PubMed: 22079502]
147. Jackson A, Nussbaum J, Phulusa J, et al. A phase II randomized controlled trial adding oral flucytosine to high-dose fluconazole with short-course amphotericin B for cryptococcal meningitis. AIDS. 2012; 26:1363–70. [PubMed: 22526517]
148. Nussbaum JC, Jackson A, Namarika D, et al. Combination flucytosine and high-dose fluconazole compared with fluconazole monotherapy for the treatment of cryptococcal meningitis: a randomized trial in Malawi. Clin Infect Dis. 2010; 50:338–44. [PubMed: 20038244]
149. Bozette SA, Larsen RA, Chiu J, et al. A placebo-controlled trial of maintenance therapy with fluconazole after treatment for cryptococcal meningitis in the Acquired Immunodeficiency Syndrome. N Engl J Med. 1991; 324:580–4. [PubMed: 1992319]
150. Vibhagool A, Sungkanuparph S, Mootsikapun P, et al. Discontinuation of secondary prophylaxis for cryptococcal meningitis in human immunodeficiency virus-infected patients treated with HAART: a prospective, multicenter, randomized study. Clin Infect Dis. 2003; 36:1329–31. [PubMed: 12746781]
151. Mussini C, Pezzotti P, Miro JM, et al. Discontinuation of maintenance therapy for cryptococcal meningitis in patients with AIDS treated with HAART: an international observational study. Clin Infect Dis. 2004; 38:565–71. [PubMed: 14765351]
152. Denning DW, Tucker RM, Hanson LH, et al. Itraconazole therapy for cryptococcal meningitis and cryptococcosis. Arch Intern Med. 1989; 149:2301–8. [PubMed: 2552949]
153. Saag MS, Cloud GA, Graybill JR, et al. A comparison of itraconazole versus fluconazole as maintenance therapy for AIDS-associated cryptococcal meningitis. National Institute of Allergy and Infectious Diseases Mycoses Study Group. Clin Infect Dis. 1999; 28:291–6. [PubMed: 10064246]
154. Perfect JR, Marr KA, Walsh TJ, et al. Voriconazole treatment for less-common, emerging or refractory fungal infections. Clin Infect Dis. 2003; 36(9):1122–31. [PubMed: 12715306]
155. Pitisuttithum P, Negroni R, Graybill JR, et al. Activity of posaconazole in the treatment of central nervous system fungal infections. J Antimicrob Chemother. 2005; 56:745–55. [PubMed: 16135526]
156. Bisson G, Molefi M, Bellamy S, et al. Early versus delayed antiretroviral therapy and cerebrospinal fluid fungal clearance in adults with HIV and cryptococcal meningitis. Clin Infect Dis. 2013; 56(8):1165–73. [PubMed: 23362285]
157. Njei B, Kongnyuy EJ, Kumar S, et al. Optimal timing for HAART initiation in patients with HIV infection and concurrent cryptococcal meningitis. Cochrane Database Syst Rev. 2013(2):CD009012.
158. Makadzange AT, Ndhlovu CE, Takarinda K, et al. Early versus delayed initiation of HAART for concurrent HIV infection and cryptococcal meningitis in sub-Saharan Africa. Clin Infect Dis. 2010; 50(11):1532–8. [PubMed: 20415574]

159. Abdool Karim S, Naidoo K, Grobler A, et al. Integration of antiretroviral therapy with tuberculosis treatment. N Engl J Med. 2011; 365(16):1492. [PubMed: 22010915]

160. Blanc F, Sok T, Laureillard D, et al. Earlier versus later start of antiretroviral therapy in HIV-infected adults with tuberculosis. N Engl J Med. 2011; 365(16):1471. [PubMed: 22010913]

161. Boulware D, Meya D, Muzoora C, et al. Timing of antiretroviral therapy after cryptococcal meningitis. N Engl J Med. 2014; 370:2487–98. [PubMed: 24963568]

162. Singh N, Lortholary O, Alexander BD, et al. Antifungal management practices and evolution of infection in organ transplant recipients with *Cryptococcus neoformans* infection. Transplantation. 2005; 80:1033–9. [PubMed: 16278582]

163. 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. [PubMed: 15909263]

164. 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. 1970; 301(3):126–31.

165. Denning DW, Armstrong RW, Lewis BH, et al. Elevated cerebrospinal fluid pressures in patients with cryptococcal meningitis and acquired immunodeficiency syndrome. Am J Med. 1991; 91:267–72. [PubMed: 1892147]

166. Rolfes M, Hullsiek K, Rhein J, et al. The effect of therapeutic lumbar punctures on acute mortality from cryptococcal meningitis. Clin Infect Dis. 2014; 59(11):1607–14. [PubMed: 25057102]

167. Newton PN, Thaile 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. [PubMed: 12203177]

168. Park MK, Hospenthal DR, Bennett JE. Treatment of hydrocephalus secondary to cryptococcal meningitis by use of shunting. Clin Infect Dis. 1999; 28:629–33. [PubMed: 10194090]

169. Velez JD, Allendorfer R, Luther M, et al. Correlation of in vitro azole susceptibility testing with in vivo response in a murine model of cryptococcal meningitis. J Infect Dis. 1993; 168:508–10. [PubMed: 8335995]

170. 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. [PubMed: 10817706]

171. Smith R, Mba-Jonas A, Tourdjman M, et al. Treatment and outcomes among patients with *Cryptococcus gattii* infections in the United States Pacific Northwest. PLoS One. 2014; 9(2):e88875. [PubMed: 24586423]

172. Nightingale SD, Cal SX, Peterson DM, et al. Primary prophylaxis with fluconazole against systemic fungal infections in HIV-positive patients. AIDS. 1992; 6:191–4. [PubMed: 1348417]

173. Chetchotisakd P, Sungkanuparph S, Thinkhamrop B, et al. A multicentre, randomized, double-blind, placebo-controlled trial of primary cryptococcal meningitis prophylaxis in HIV-infected patients with severe immune deficiency. HIV Med. 2004; 5(3):140–3. [PubMed: 15139978]

174. Chang L, Phipps W, Kennedy G, et al. Antifungal interventions for the primary prevention of cryptococcal disease in adults with HIV. Cochrane Database Syst Rev. 2005; (3):CD004773. [PubMed: 16034947]

175. French N, Gray K, Watera C, et al. Cryptococcal infection in a cohort of HIV-1 infected Ugandan adults. AIDS. 2002; 16:1031–8. [PubMed: 11953469]

176. McKenney J, Bauman S, Neary B, et al. Prevalence, correlates and outcomes of cryptococcal antigen positivity among patients with AIDS, United States, 1986–2012. Clin Infect Dis. 2015; 60(6):959–65. [PubMed: 25422390]

177. Jarvis JN, Lawn SD, Vogt M, et al. Screening for cryptococcal antigenemia in patients accessing an antiretroviral treatment program in South Africa. Clin Infect Dis. 2009; 48:856–62. [PubMed: 19222372]

178. Meya D, Manabe Y, Castelnuovo B, et al. Cost-effectiveness of serum cryptococcal antigen screening to prevent deaths among HIV-infected persons with a $CD4^+$ cell count of < 100 cells/

μL who start HIV therapy in resource-limited settings. Clin Infect Dis. 2010; 51(4):448–55. [PubMed: 20597693]

179. Kaplan J, Vallabhaneni S, Smith R, et al. Cryptococcal antigen screening and early antifungal treatment to prevent cryptococcal meningitis: a review of the literature. J Acquir Immune Defic Syndr. 2015; 68:S331–9. [PubMed: 25768872]
180. Smith R, Nguyen T, Ha H, et al. Prevalence of cryptococcal antigenemia and cost-effectiveness of a cryptococcal antigen screening program – Vietnam. PLoS One. 2013; 8(4):e62213. [PubMed: 23626792]
181. World Health Organization. Rapid advice: diagnosis, prevention and management of Cryptococcal disease in HIV-infected adults, adolescents and children. Geneva (Switzerland): World Health Organization; 2011.
182. Rajasinham R, Boulware D. Reconsidering cryptococcal antigen screening in the US among persons with CD4 < 100 cells/mcl. Clin Infect Dis. 2012; 55:1742–4. [PubMed: 22918997]
183. Singh N, Dromer F, Perfect JR, et al. Cryptococcosis in solid organ transplant recipients: current state of the science. Clin Infect Dis. 2008; 47:1321–7. [PubMed: 18840080]
184. Devi SJ, Scheerson R, Egan W, et al. *Cryptococcus neoformans* serotype A glucuronoxylomannan protein conjugate vaccines: synthesis, characterization, and immunogenicity. Infect Immun. 1991; 59:3700–7. [PubMed: 1716613]
185. Mukherjee J, Zuckier LS, Scharff MD, et al. Therapeutic efficacy of monoclonal antibodies to *Cryptococcus neoformans* glucuronoxylomannan alone and in combination with amphotericin B. Antimicrob Agents Chemother. 1994; 38:580–7. [PubMed: 8203858]
186. Wormley F, Perfect J, Steele C, et al. Protection against cryptococcosis by using a murine gamma interferon-producing *Cryptococcus neoformans* strain. Infect Immun. 2007; 75:1453–63. [PubMed: 17210668]
187. Jarvis J, Meintjes G, Rebe K, et al. Adjunctive interferon-g immunotherapy for the treatment of HIV-associated cryptococcal meningitis: a randomized controlled trial. AIDS. 2012; 26(9):1105–13. [PubMed: 22421244]
188. Pappas P, Bustamante B, Ticona E, et al. Recombinant interferon-gamma 1b as adjunctive therapy for AIDS-related acute cryptococcal meningitis. J Infect Dis. 2004; 1889(12):2185–91.
189. Isiodras S, Samonis G, Boumpas D, et al. Fungal infections complicating tumor necrosis factora blockade therapy. Mayo Clin Proc. 2008; 83(2):181–94. [PubMed: 18241628]
190. Lin Y, Shiau S, Fang C. Risk factors for invasive Cryptococcus neoformans diseases: a case-control study. PLoS One. 2015; 10(3):e0119090. [PubMed: 25747471]
191. Singh N, Perfect JR. Immune reconstitution syndrome associated with opportunistic mycoses. Lancet Infect Dis. 2007; 7(6):395–401. [PubMed: 17521592]

KEY POINTS

- Cryptococcosis is a major invasive fungal infection that is capable of widespread disease outbreaks in both immunocompromised and apparently immunocompetent hosts.
- Molecular advances continue to enhance our understanding of *Cryptococcus* and provide insight into its evolution into a pathogen of global importance.
- Diagnosis has improved with the introduction of point-of-care diagnostic assays.
- Screening and preemptive antifungal therapy offer great promise in making a significant impact in this highly deadly opportunistic mycosis.

Box 1

Suggested diagnostic criteria for the immune reconstitution inflammatory syndrome

New appearance or worsening of any of the following:

Clinical or radiographic manifestations consistent with an inflammatory process:

> **Central nervous system:** Contrast-enhancing lesions on neuroimaging (computed tomography or MRI); cerebrospinal fluid pleocytosis (ie, >5 white blood cell count per μL); increased intracranial pressure (ie, opening pressure of ≥20 mm H_20), with or without hydrocephalus.
>
> **Pulmonary:** Nodules, cavities, masses or pleural effusions.
>
> **Other:** Lymphadenopathy, skin, soft tissue, osteoarticular lesions.

Histopathology showing granulomatous lesions.

Symptoms occurring during receipt of appropriate antifungal therapy[a] that cannot be explained by a newly acquired infection or another process (neoplasm, etc).

Negative results of cultures, or stable or reduced biomarkers for the initial fungal pathogen during the diagnostic workup for the inflammatory process.

All 3 criteria must be present for a positive diagnosis.

[a] Exclude intrinsic and de novo drug resistance, and suboptimum drug concentrations.

Adapted from Sun H, Alexander B, Huprikar S, et al. Predictors of immune reconstitution syndrome in organ transplant recipients with cryptococcosis: implications for the management of immunosuppression. Clin Infect Dis 2015;60(1):36–44; and Singh N and Perfect JR. Immune reconstitution syndrome associated with opportunistic mycoses. Lancet Infect Dis 2007; 7:398.

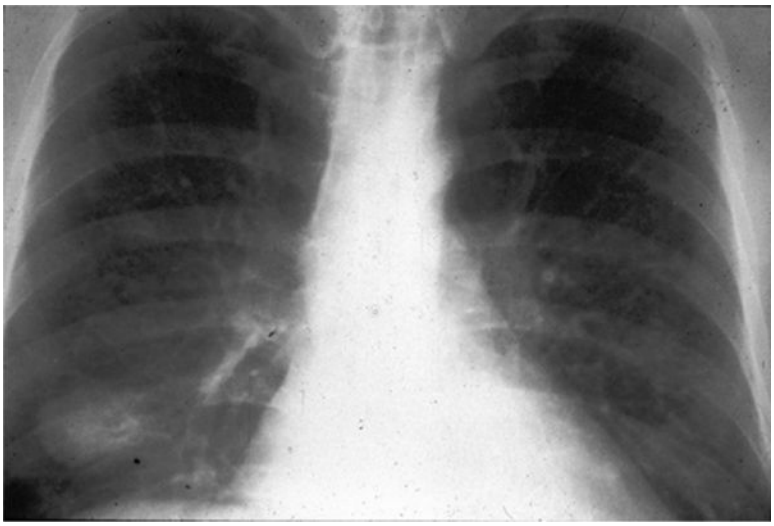

Fig. 1.

Solitary pulmonary nodule. In an asymptomatic patient with isolated pulmonary cryptococcosis. (*Courtesy of* J. R. Perfect, MD, Durham, NC.)

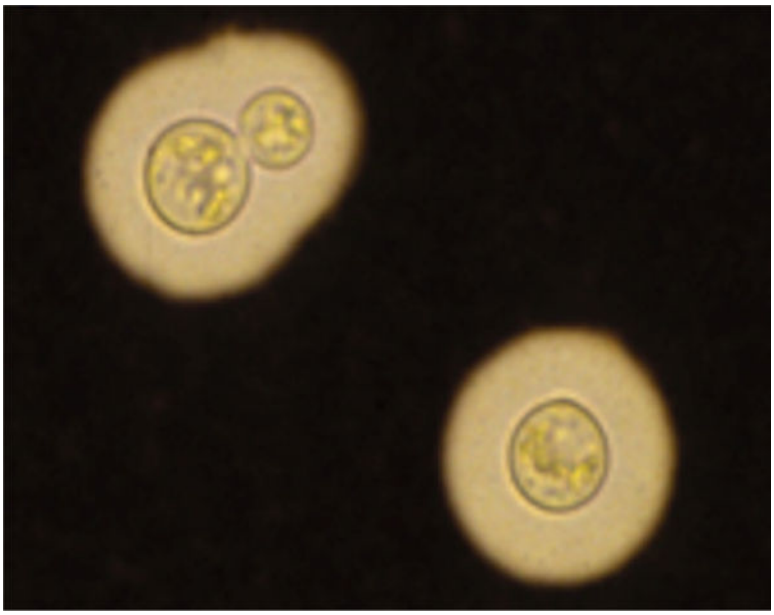

Fig. 2.

India ink staining. Encapsulated yeast seen on India ink preparation of cerebrospinal fluid in a patient with cryptococcal meningitis. (*Courtesy of* J. R. Perfect, MD, Durham, NC.)

Table 1

Current classification of pathogenic *Cryptococcus* species

Serotype	Species and Varieties	Molecular Types
A	*C neoformans* var. *grubii*[a]	VN I, VN II
B	*C gattii*	VG I, VG II, VG III, VG IV
C	*C gattii*	VG I, VG II, VG III, VG IV
D	*C neoformans* var. *neoformans*	VN IV
AD	*C neoformans*	VN III

[a]Responsible for the vast majority of disease owing to C neoformans worldwide.

Adapted from Hagen F, Khayhan K, Theelen B, et al. Recognition of seven species in the *Cryptococcus gatti/Cryptococcus neoformans* species complex. Fungal Genet Biol 2015;78:17.

Table 2

Proposed taxonomy changes for the *Cryptococcus neoformans*/*C gattii* complex

Current Species Name	Genotype by RFLP	Proposed Species Name
C neoformans var. *grubii*	VNI	*C neoformans*
	VNII	
	VNIII	
C neoformans var. *neoformans*	VNIV	*C deneoformans*
C neoformans intervariety hybrid	VNIII	*C neoformans* × *C deneoformans* hybrid
C gattii	VGI	*C gattii*
	VGIII	*C bacillisporus*
	VGII	*C deuterogattii*
	VGIV	*C tetragattii*
	VGIV/VGIIIc	*C decagattii*
C neoformans var. *neoformans* × *C gattii* AFLP4/VGI hybrid	—	*C deneoformans* × *C gattii* hybrid
C neoformans var. *grubii* × *C gattii* AFLP4/VGI hybrid	—	*C neoformans* × *C gattii* hybrid
C neoformans var. *grubii* × *C gattii* AFLP6/VGII hybrid	—	*C deneoformans* × *C deuterogattii* hybrid

Adapted from Hagen F, Khayhan K, Theelen B, et al. Recognition of seven species in the *Cryptococcus gatti/Cryptococcus neoformans* species complex. Fungal Genet Biol 2015;78:17.

Table 3

Risk factors for *Cryptococcus* infection

HIV infection	**Rheumatologic diseases[a] Systemic lupus erythematosus Rheumatoid arthritis**
Corticosteroid and/or immunosuppressive therapies	Idiopathic $CD4^+$ lymphopenia
Solid organ transplantation[a]	Chronic liver disease (decompensated)[b]
Malignant and lymphoproliferative disorders[a,b]	Renal failure and/or peritoneal dialysis
Sarcoidosis	Hyper-IgM syndrome or hyper-IgE syndrome
Treatment with monoclonal antibodies (etanercept, infliximab, alemtuzumab)	*Diabetes mellitus*[c]
Anti-GM CSF antibodies	—

Abbreviations: GM CSF, granulocyte macrophage colony stimulating factor; HIV, human immunodeficiency virus; Ig, immunoglobulin.

[a] Immunosuppression for these conditions may influence risk.

[b] Poor prognosis especially among patients with hematologic malignancy.[32]

[c] Historically considered a risk factor but may reflect the frequency of condition rather than specific risk to an individual. Not found to be a risk factor in.[190,191]

Adapted from Casadevall A, Perfect JR. Cryptococcus neoformans. Washington, DC: ASM Press; 1998.

Table 4

Treatment recommendations for HIV-associated cryptococcal meningoencephalitis

	Duration
Induction therapy	
Primary regimen	
AmBd (0.7–1 mg/kg/d) plus flucytosine (5-FC) (100 mg/kg/d)[a]	2 wk
Alternative regimens[b]	
If 5-FC intolerant or unavailable: AmBd (0.7–1 mg/kg/d) or L-AMB[c] (3–4 mg/kg/d) or ABLC (5 mg/kg/d)	4–6 wk
AmBd (0.7–1 mg/kg/d) plus fluconazole (800 mg/d)	2 wk
Fluconazole (≥800 mg/d, preferably 1200 mg/d) plus 5-FC (100 mg/kg/d)	6 wk
Fluconazole (800–2000 mg/d, preferably 1200 mg/d)	10–12 wk
Itraconazole (200 mg BID)	10–12 wk
Consolidation therapy	
Fluconazole (400 mg/d)	8wk[d]
Maintenance or suppressive therapy	
Fluconazole (200 mg/d)	≥1 y[e]
Alternative reqimens[a]	
Itraconazole (200 mg BID)	≥1 y[e]
AmBd (1 mg/kg IV per week)	≥1 y

Abbreviations: 5-FC, flucytosine; ABLC, amphotericin B lipid complex; AmBd, amphotericin B deoxycholate; BID, twice daily; L-AMB, liposomal amphotericin B.

[a] L-AMB, 3–4 mg/kg/d or AmB lipid complex (ABLC; 5 mg/kg/d) for patients predisposed to renal dysfunction.

[b] Can be considered as alternative regimen when primary regimen not available but not encouraged as equivalent substitutes.

[c] L-AMB can be safely administered in doses as high as 6 mg/k/d.

[d] Initiate highly active antiretroviral therapy approximately 4 weeks after beginning antifungal regimen.

[e] After 1 year of therapy, if successful response to antiretroviral drugs (CD4 count ≥ 100 and viral load low or undetectable for >3 months), can consider discontinuation of antifungal therapy. Consider reinstitution if CD4 count is <100.

Adapted from Perfect JR, Dismukes WE, Dromer F, et al. Clinical practice guide lines for the management of cryptococcal disease: 2010 update by the Infectious Disease Society of America. Clin Infect Dis 2010;50:291–322.