Cryptococcosis

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Introduction

Cryptococcosis is an infectious disease with a wide range of clinical presentations caused by pathogenic encapsulated yeasts in the genus *Cryptococcus*. Currently, there are two species of these fungi that commonly cause disease in humans: *Cryptococcus neoformans,* which causes cryptococcosis in both immunocompetent and immunocompromised hosts, and *Cryptococcus gattii*, which is primarily a pathogen in apparently immunocompetent patients but can also cause disease in the immunocompromised. *C. neoformans* was first identified as a human pathogen in 1894 by two German physicians, Otto Busse and Abraham Buschke, when they described a circular yeast-like microorganism in a lesion on the tibia of a woman; the microorganism was initially named *Saccharomyces hominis* [1]. The name *C. neoformans* has been consistently adopted in both the mycology and medical literature since 1950 [2]. In the mid-1970s, when Kwon-Chung discovered two mating types of *C. neoformans* that produced fertile basidiospores, the organisms were subsequently separated into two varieties, var. *neoformans* (serotypes A and D) and var. *gattii* (serotypes B and C). These two varieties were recently separated into two species, *C. neoformans* and *C. gattii,* based on their genetic background and phylogenetic diversity, as proposed by Kwon-Chung in 2002 [3]. It is possible, as more molecular information is gathered from genome sequencing, that *C. neoformans* var. *neoformans* (serotype D) and *C. neoformans* var. *grubii* (serotype A) will be divided into separate species as well as other cryptic species.

The incidence of cryptococcosis began to rise in the late 1970s. Early case reports of cryptococcal infections were primarily associated with cancer, autoimmune diseases, organ transplantation, and receipt of corticosteroids as these immunocompromised populations expanded [4]. A major surge in new cases of cryptococcosis occurred during the first two decades of the HIV pandemic, when cryptococcal infection was an important opportunistic infection (OI) in all parts of the world. Furthermore, around 2000, *C. gattii* strains (previously geographically restricted to tropical and subtropical regions) caused a localized outbreak of cryptococcosis in apparently immunocompetent individuals on Vancouver Island [5]. This has increased recognition that these fungi can exploit new geographical environments and cause disease in both immunocompromised and apparently immunocompetent hosts. Despite the development of highly active antiretroviral therapy (HAART), which has decreased the rate of HIV-related cryptococcosis in developed countries, the burden of cryptococcal infection is still very high in developing countries and in those individuals without access to health care. It has been estimated that there are a million cases per year with more than 600,000 deaths due to cryptococcosis worldwide [6].

Etiologic Agents

Cryptococcus is a genus of heterobasidiomycetous fungi containing more than 30 species. However, the common pathogenic organisms of cryptococcosis currently consist of two species, which can further be classified into three varieties, five serotypes (based on structural differences in the polysaccharide capsule), and eight molecular subtypes (Table 15.1). *C. neoformans* is classified into serotype A and D and the hybrid strain AD, whereas serotype B and C strains are classified as *C. gattii*. Serotype A strains have been further classified as *C. neoformans* var. *grubii* and serotype D strains are named *C. neoformans* var. *neoformans*. Recently, both *C. neoformans* and *C. gattii* have been further divided into molecular subtypes for each species, VN I–IV, VN B and VG I–IV, respectively.

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Table 15.1 Classification of *Cryptococcus neoformans* and *Cryptococcus gattii*

Serotype	Species and varieties	Molecular types
A	*C. neoformans* var. *grubii*	VN I, VN II, VN B
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

The life cycle of *C. neoformans* and *C. gattii* involve asexual (yeast) and sexual (basidiospores/hyphae) forms. The asexual form is the encapsulated yeast that reproduces by narrow-based budding and is found most commonly in clinical specimens, whereas the sexual stage, which exists in one of two mating types, "alpha" or "a," is observed only under certain conditions, resulting in meiosis to form basidiospores. The vast majority of clinical infections and environmental isolates are caused by "alpha" mating-type locus strains. Since the sexual stage of *C. neoformans* and *C. gattii* has been described, their teleomorphs were named *Filobasidiella neoformans* and *Filobasidiella bacillospora,* respectively.

C. neoformans and *C. gattii* usually appear as white-to-cream, opaque, and mucoid colonies that grow to several millimeters in diameter on the most routine agar within 48–72 h. With some strains, a few colonies occasionally develop sectors with different pigmentation or different morphologies (i.e., wrinkled, smooth, mucoid). Both cryptococcal species will grow readily on most fungal culture media without cycloheximide at 30–37 °C in aerobic conditions. However, *C. neoformans* is generally more thermotolerant than *C. gattii,* and, within this species, serotype A is generally more tolerant than serotype D strains. In addition to their ability to grow at 37 °C, the yeast produce a thick shedding polysaccharide capsule, melanin pigments, and the enzymes urease and phospholipase, which allow *Cryptococcus* to be readily identified from other yeasts. These are also considered to be yeast virulence factors.

Epidemiology

Cryptococcosis was considered an uncommon infection prior to the AIDS epidemic, associated with malignancies, organ transplantation, and certain immunosuppressive treatments. Beginning in the early 1980s, the incidence of cryptococcosis increased significantly and between 6 and 10 % of persons with AIDS developed cryptococcosis [7, 8]. In fact, HIV/AIDS was found to be associated with 80 % of cryptococcosis cases worldwide. Cryptococcal infection is a major OI in HIV-infected patients as the CD4$^+$ cell count falls below 100 cells/μl. Following widespread implementation of HAART, the incidence of cryptococcosis among

Table 15.2 Predisposing factors of cryptococcosis

HIV infection
Malignancies[a] (e.g., Hodgkin's disease, other lymphomas, and chronic lymphocytic leukemia)
Lymphoproliferative disorders[a]
Idiopathic CD4$^+$ T cell lymphopenia
Rheumatologic or immunologic diseases[a]
Sarcoidosis
Systemic lupus erythematosus
Rheumatoid arthritis
Hyper-IgM syndrome or hyper-IgE syndrome
Monoclonal antibodies (etanercept, infliximab, alemtuzumab)
Corticosteroid and/or immunosuppressive therapies
Diabetes mellitus
Solid organ transplantation[a]
Chronic pulmonary diseases
Renal failure and/or peritoneal dialysis
Chronic liver diseases[b]

IgE immunoglobulin E, *IgG* immunoglobulin G
[a] Immunosuppressive therapies add to the risk
[b] Poor prognosis

patients with HIV/AIDS has fallen significantly in most developed nations. The incidence of cryptococcal infection in persons not infected with HIV has remained stable during this time. Moreover, in developing nations with limited access to HAART, the prevalence of and morbidity and mortality associated with cryptococcosis remains unacceptably high, accounting for up to 600,000 deaths per year. Besides HIV infection, other risk factors for acquiring cryptococcal infections include many conditions that result in an immunocompromised status (Table 15.2). Although both *C. neoformans* and *C. gattii* can cause cryptococcosis in apparently normal hosts, the percentage of *C. gattii* infections causing disease in such patients is significantly higher than for *C. neoformans*.

C. neoformans is found throughout the world in association with excreta from certain birds such as pigeons and in tree hollows. *C. gattii* is commonly associated with several species of eucalyptus and other trees [9]. While the link between the environmental source of infection and cryptococcosis cases is not precise, there is evidence to suggest an increased risk of cryptococcosis and asymptomatic cryptococcal antigenemia following intense bird exposures. Recently, there has been a strong link between the *C. gattii* outbreak in humans on Vancouver Island and common environmental yeast exposures. Although these fungi can be detected in endobronchial specimens from humans without disease (colonization), clinicians should be alert for subclinical disease or potential for disease when *Cryptococcus* is isolated from any clinical specimen.

Approximately 95 % of cryptococcal infections are caused by serotype A strains (*C. neoformans* var. *grubii*) with the remaining 4–5 % of infections caused by serotype D (*C. neoformans* var. *neoformans*) or serotype B and C strains (*C. gattii*). Whereas *C. neoformans* serotype A is found worldwide, serotypes B and C are found primarily in

tropical and subtropical regions such as southern California, Hawaii, Brazil, Australia, Southeast Asia, and central Africa (and more recently identified in temperate climates such as Vancouver Island and the Pacific Northwest region of the USA), and serotype D is predominantly found in European countries (Table 15.3) [10]. In Australia and New Zealand, serotypes B and C caused up to 15 % of all cases of cryptococcosis cases in one study, but serotype A remains the predominant serotype even in these endemic areas [11]. To date, only *C. gattii* strains have been reported to cause a widespread defined outbreak of disease [5].

Table 15.3 Distribution of *C. neoformans* and *C. gatti*

Cryptococcus species	Primary areas of distribution
C. neoformans var. *grubii* serotype A	Worldwide; pigeon guano, tree hollows
C. gattii	Tropical and subtropical regions: southern California, Hawaii, Brazil, Australia, Southeast Asia, and central Africa; eucalyptus trees, firs, and oak trees
C. neoformans var. *neoformans* serotype D	Europe: Denmark, Germany, Italy, France, and Switzerland; less common in the environment than serotype A

Pathogenesis and Immunology

Cryptococcosis occurs primarily by inhalation of the infectious propagules, either dehydrated (poorly encapsulated) yeasts or basidiospores, into pulmonary alveoli. Direct inoculation into tissue due to trauma can be a portal of entry in occasional cases and, potentially, yeast may enter through the gastrointestinal tract. After the yeasts are inhaled into the lungs of a susceptible host, they encounter alveolar macrophages, and other inflammatory cells are recruited through release of cytokines and chemokines such as interleukin (IL)-12, IL-18, monocyte chemotactic protein (MCP)-1, and macrophage inflammatory protein (MIP)-1α. Cryptococcal infection primarily involves granulomatous inflammation, which is a result of a helper T cell (Th1) response with cytokines including tumor necrosis factor, interferon-γ, and IL-2 [12]. In many circumstances, the yeasts remain dormant (yet viable) in hilar lymph nodes or pulmonary foci of an asymptomatic individual for years and then disseminate outside those complexes when local immunity is suppressed, similar to that which is observed in cases of reactivation tuberculosis or histoplasmosis [10]. In a patient with severely compromised cellular immunity, the yeasts reactivate and proliferate at the site of infection and then disseminate to other sites causing progressive clinical symptoms.

Recent advances in the molecular biology of *Cryptococcus* have confirmed several virulence factors. The three classical virulence factors of *C. neoformans* include: capsule formation, melanin pigment production, and the ability to grow well at 37 °C [9, 12]. The prominent antiphagocytic polysaccharide capsule, which is composed of glucuronoxylomannan (GXM), is unique to *Cryptococcus* species and is considered an essential virulence factor that has multiple effects on host immunity. In addition, *C. neoformans* possesses an enzyme that catalyzes the conversion of diphenolic compounds to form melanin, which may have a biological role to protect the yeasts from host oxidative stresses and which may partially explain the organism's neurotropism. Finally, its ability to grow at 37 °C is a basic part of the virulence composite for most of the human pathogenic fungi including *Cryptococcus,* as molecular studies have linked high-temperature growth with certain signaling pathways and enzymes that this yeast has acquired or adapted over time in order to enhance its pathogenicity. Other virulence factors include phospholipase and urease production and multiple enzymes associated with protection against oxidative stresses.

Clinical Manifestations

C. neoformans and *C. gattii* have a predilection for establishing clinical disease in the lungs and central nervous system (CNS). Other organs that may be involved in cryptococcosis include skin, prostate, eyes, bone, and blood [2, 8, 10, 13]. In fact, this yeast may cause disease in any organ of the human body, and widely disseminated cryptococcal infection can affect multiple organs in severely immunosuppressed patients (Table 15.4).
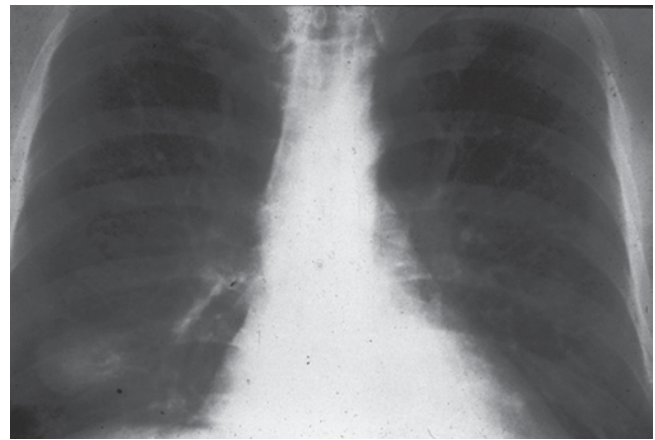
Pulmonary Infection

The respiratory tract serves as the most important portal of entry for this yeast, and thus there are many clinical manifestations of pulmonary cryptococcosis, ranging from asymptomatic transient or chronic colonization of the airways or simply a pulmonary nodule on radiograph to life-threatening fungal pneumonia with acute respiratory distress syndrome (ARDS) [2, 8]. In a normal host with cryptococcal infection, asymptomatic pulmonary cryptococcosis can occur in about one third of patients with pulmonary infection and patients may present to care with only an abnormal chest radiograph. The most common radiologic findings of cryptococcosis include well-defined single or multiple noncalcified nodules (Fig. 15.1) and pulmonary infiltrates (Fig. 15.2), but other less frequent radiographic findings include pleural effusions, hilar lymphadenopathy, and lung cavitation. Patients with pulmonary cryptococcosis can present with symptoms of acute onset of fever, productive cough, respiratory distress, chest pain, and weight loss [14]. The outbreak of *C.*

Table 15.4 Clinical manifestations of cryptococcosis. (Adapted from Casadevall, A, Perfect, JR. *Cryptococcus neoformans*. Washington: ASM Press; 1998: 409 [2])

Organs	Common clinical manifestations
Central nervous system	Acute/subacute/chronic meningoencephalitis Cryptococcomas (abscesses) Spinal cord granuloma Chronic cognitive impairment (sequelae of hydrocephalus)
Lung	Asymptomatic airway colonization Pulmonary nodule(s) Hilar or mediastinal lymphadenopathy Lobar/interstitial infiltrates Miliary infiltrates Lung cavities Endobronchial lesions Pleural effusion/empyema Pneumothorax Acute/subacute pneumonia Acute respiratory distress syndrome
Skin	Papules with central ulceration (molluscum contagiosum-like) Subcutaneous abscesses Nodules/papules Cellulitis Draining sinuses Ulcers
Eye	Papilledema Endophthalmitis Optic nerve atrophy Chorioretinitis Keratitis Paresis of extraocular muscles
Genitourinary tract	Prostatitis Cryptococcuria Renal abscess Genital lesions
Bone and joints	Osteolytic lesion(s) Arthritis (acute/chronic)
Cardiovascular system	Cryptococcemia Endocarditis (native/prosthetic) Mycotic aneurysm Myocarditis Pericarditis
Other organs	Myositis Peritonitis Hepatitis Nodular/ulcerative GI mucosal lesions Pancreatic mass Breast abscess Adrenal mass and adrenal insufficiency Thyroiditis or thyroid mass Sinusitis Salivary gland enlargement

GI gastrointestinal

Fig. 15.1 Chest radiograph of pulmonary cryptococcosis presents as a single nodule in the lung at right lower lung field. (From A. Casadevall and J. R. Perfect, *Cryptococcus neoformans*, ASM Press, 1998. Reprinted with permission from Oxford University Press)
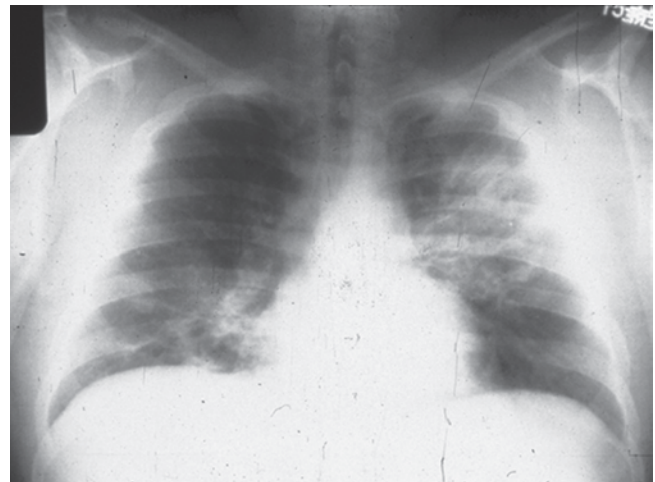
Fig. 15.2 Chest radiograph of pulmonary cryptococcosis presents as left lobar infiltrates. (From A. Casadevall and J. R. Perfect, *Cryptococcus neoformans*, ASM Press, 1998. Reprinted with permission from Oxford University Press)

gattii infections in Vancouver Island included several cases of severe symptomatic pulmonary cryptococcosis in apparently immunocompetent individuals. In an immunocompromised patient, especially those with HIV infection, cryptococcal pneumonia is usually symptomatic and can progress rapidly to ARDS, even in the absence of CNS involvement. Most immunocompromised patients with cryptococcal in-

fection, however, present with CNS rather than pulmonary symptoms. In fact, more than 90 % of HIV/AIDS patients with cryptococcal infection already have CNS cryptococcosis at the time of diagnosis, many of whom will have a paucity of respiratory complaints. The findings in chest radiographs of immunocompromised patients with pulmonary cryptococcosis are the same as those in immunocompetent patients, but alveolar and interstitial infiltrates tend to be more frequent and imaging can mimic *Pneumocystis* pneumonia. Accelerated presentations of cryptococcal pneumonia are more common among immunocompromised patients. In pulmonary cryptococcosis, if the infection is confined to the lung, serum cryptococcal polysaccharide antigen is usually negative. However, while a positive serum polysaccharide

antigen may indicate the dissemination of the yeast from the lung, it does not confirm CNS involvement. In immunocompromised individuals with pulmonary cryptococcosis, a lumbar puncture to rule out CNS disease should be considered regardless of the patient's symptoms or serum polysaccharide antigen test results. The only setting in which screening a lumbar puncture may not necessarily need to be performed in a patient with *Cryptococcus* isolated from the lung is in the asymptomatic, immunocompetent patient with disease that appears to be limited to the lungs.

CNS Infection

Clinical manifestations of CNS cryptococcosis include headache, fever, cranial neuropathy, alteration of consciousness, lethargy, memory loss, and signs of meningeal irritation [2, 8]. These findings are usually present for several weeks and therefore cause a clinical syndrome of subacute meningitis or meningoencephalitis. However, on some occasions, patients can present more acutely or lack typical features including headache. In HIV-infected patients with CNS cryptococcosis, the burden of fungal organisms in the CNS is usually high. Therefore, these patients may have a shorter onset of signs and symptoms, higher cerebrospinal fluid (CSF) polysaccharide antigen titers and intracranial pressures (ICPs), and slower CSF sterilization after starting antifungal treatment.

Different species may produce differences in clinical manifestations. For instance, one species may have a predilection to cause disease in brain parenchyma rather than the meninges. In certain areas of the world, *C. gattii* tends to cause cerebral cryptococcomas (Fig. 15.3) and/or hydrocephalus with or without large pulmonary mass lesions more frequently than *C. neoformans*. These patients with brain parenchymal involvement usually have high ICP and cranial neuropathies, and respond poorly to antifungal therapy.

Skin Infection

Cutaneous infections are the third most common clinical manifestations of cryptococcosis. Patients can manifest several types of skin lesions. One common skin lesion is a papule or maculopapular rash with central ulceration that may be described as "molluscum contagiosum-like." These lesions are indistinguishable from those due to other fungal infections including *Histoplasma capsulatum*, *Coccidioides immitis*, and *Penicillium marneffei*. Other cutaneous lesions of cryptococcosis include acneiform lesions, purpura, vesicles, nodules, abscesses, ulcers (Fig. 15.4), granulomas, pustules, plaques, draining sinus, and cellulitis. Because there are many skin manifestations in cryptococcosis that mimic
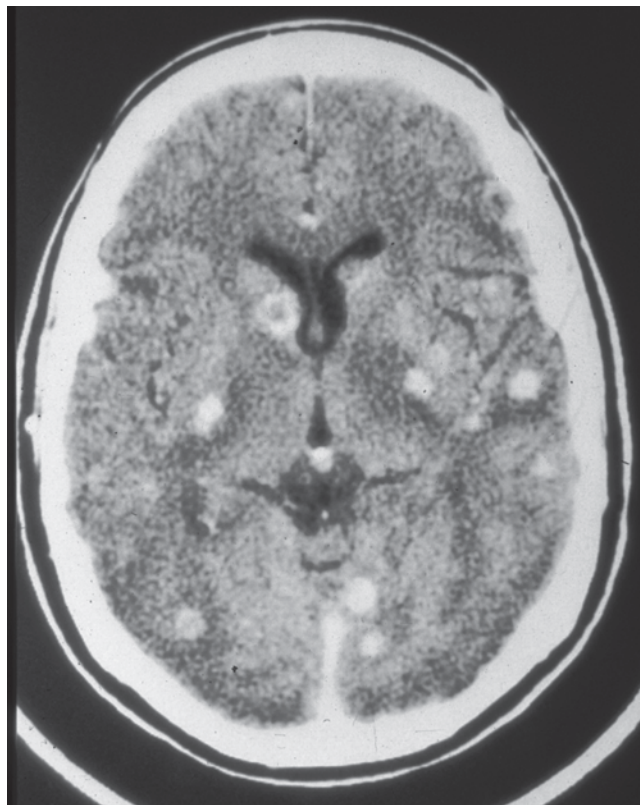
Fig. 15.3 CT scan of the brain showing multiple cryptococcomas in an apparently normal host. *CT* computed tomography. (From A. Casadevall and J. R. Perfect, *Cryptococcus neoformans*, ASM Press, 1998. Reprinted with permission from Oxford University Press)

Fig. 15.4 Skin ulceration and cellulitis as cutaneous cryptococcosis

other infectious as well as malignant conditions, skin biopsy with culture and histopathology are essential for definitive diagnosis. Skin lesions of cryptococcosis usually represent disseminated cryptococcal infection. Primary cutaneous cryptococcosis is very rare and is usually associated with skin injury and direct inoculation of the yeasts. Solid organ transplant (SOT) recipients on tacrolimus seem to be more

likely to develop skin, soft-tissue, and osteoarticular infections due to *Cryptococcus* [15]. Tacrolimus has anti-cryptococcal activity at high temperatures, but loses this activity as environmental temperatures decrease; this may in part explain the increased frequency of cutaneous cryptococcosis in these patients. Despite this series of patients, however, the most common site of disseminated infection in SOT recipients still remains the CNS, including patients receiving tacrolimus.

Prostate Infection

Prostatic cryptococcosis is usually asymptomatic, and the prostate gland is considered to be a sanctuary site for this yeast. The prostate may serve as an important reservoir for relapse of cryptococcosis in patients with a high fungal burden [16]. Latent *C. neoformans* infection has even been recognized to disseminate to the bloodstream during urological surgery on the prostate [17]. Cultures of urine or seminal fluid may still be positive for *Cryptococcus* after initial antifungal treatment of cryptococcal meningitis in AIDS patients [18], strongly supporting the need for prolonged antifungal treatment to clear the prostate in these severely immunocompromised patients.

Eye Infection

In the early reports of cryptococcal meningitis before the AIDS epidemic, ocular signs and symptoms were noted in approximately 45 % of cases [19]. The most common manifestations were ocular palsies and papilledema. In the present HIV era, several other manifestations of ocular cryptococcosis have been identified, including the presence of extensive retinal lesions with or without vitritis, which can lead to irreversible blindness. Furthermore, catastrophic loss of vision without evidence for endophthalmitis has also been reported [20]. Visual loss may be due to one of two pathogenic processes. The first is caused by infiltration of the optic nerve with the yeasts, producing rapid visual loss with few effective treatments. The second is due to increased ICP and compression of the ophthalmic artery. In this setting, patients have slower visual loss and treatment with serial lumbar punctures or ventricular shunts can prevent or slow down visual loss.

Infection at Other Body Sites

In addition to lung, CNS, skin, prostate, and eye, *C. neoformans* can cause disease in many other organs (Table 15.4). Cryptococcemia can occur in severely immunosuppressed patients but rarely causes endocarditis. Bone involvement
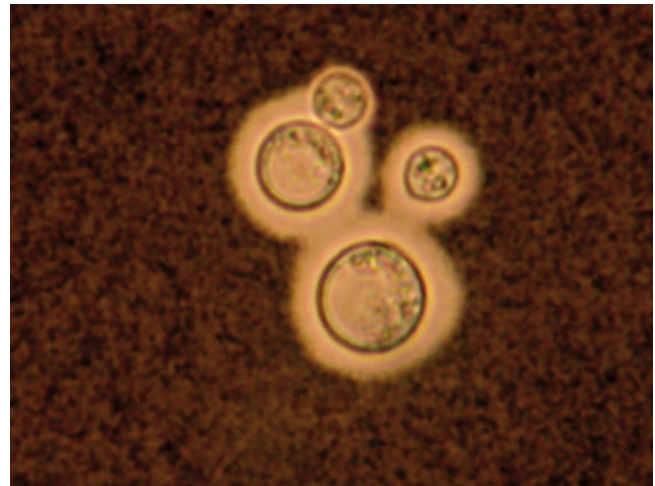
Fig. 15.5 India ink preparation showing budding encapsulated yeasts of *Cryptococcus neoformans*

of cryptococcosis typically presents as one or more circumscribed osteolytic lesions in any bone of the body, occasionally associated with "cold" soft-tissue abscesses, and has been associated with sarcoidosis. Bone marrow infiltration can be observed in severely immunocompromised hosts. Cryptococcal peritonitis [21] and cryptococcuria are also reported in several case series. Any organ of the human body can be a site of cryptococcal infections.

Diagnosis

There are several methods used for the diagnosis of cryptococcosis. These techniques include direct examination of the fungus in body fluids, histopathology of infected tissues, serological studies, and culture of body fluids or tissues. Molecular methods, while available, are not currently used in routine clinical practice.

Direct Examination

The most rapid method for diagnosis of cryptococcal meningitis is direct microscopic examination for encapsulated yeasts by an 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. It is easily distinguished in a colloidal medium of India ink when mixed with CSF (Fig. 15.5). Approximately 1–5 mL of specimen is recommended for use in the India ink preparation. India ink examination can detect encapsulated yeasts in a CSF specimen with a threshold between 10^3 and 10^4 colony-forming units of yeasts/mL of fluid. The sensitivity of the India ink preparation technique is 30–50 % in non-AIDS-related cryptococcal meningitis and

Fig. 15.6 Mouse tissues stained with various stains used to identify cryptococcal infection. *Upper left* panel is of brain stained with H&E showing meningoencephalitis with encapsulated yeast cells of *Cryptococcus neoformans*. The *upper right* panel is of kidney stained with GMS. The *middle left* panel demonstrates lung stained with Mayer's mucicarmine. Note *orange-red* staining of polysaccharide capsular material of *C. neoformans*. The *middle right* panel is liver tissue stained with PAS. Lung stained with Alcian blue stain is seen in the *bottom left* panel. Lung stained by Fontana-Masson method is seen in the *bottom right*. Melanin pigment in the cell wall of *C. neoformans* stains dark with this stain. *GMS* Gomori methenamine silver, *H&E* hematoxylin and eosin, *PAS* periodic acid-Schiff. (Courtesy of Dr. W. A. Schell)
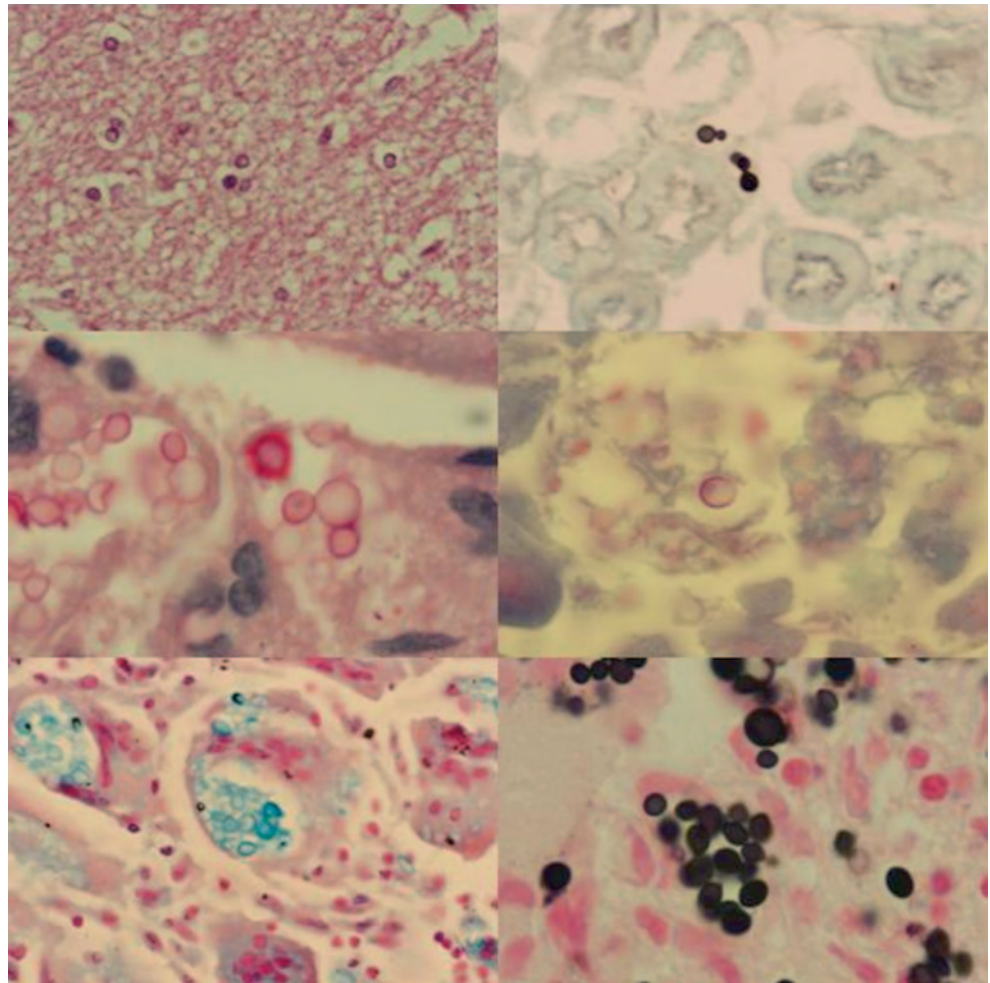
up to 80 % in AIDS-related disease. Some false-positive results can be found from intact lymphocytes, myelin globules, fat droplets, and other tissue cells. Also, dead yeast cells can remain in the CSF and be visualized by India ink preparation for varying periods of time during and after appropriate antifungal treatment. This is a limitation of direct microscopy of CSF during the management of cryptococcal meningitis [22].

Cytology and Histopathology

Cryptococcus can be identified by histological staining of tissues from lung, skin, bone marrow, brain, or other organs [23]. Histopathological staining of centrifuged CSF sediment is more sensitive for rapid diagnosis of cryptococcal meningitis than the India ink method [24]. Peritoneal fluid from chronic ambulatory peritoneal dialysis, seminal fluid, bronchial wash, or bronchoalveolar lavage fluid can also be used for cytology preparations in the diagnosis of cryptococcal infections, whereas India ink preparations from these body fluids are difficult to interpret [25, 26]. Fine needle

aspiration for cytology of peripheral lymph nodes, adrenal glands, or vitreous aspiration; percutaneous transthoracic biopsy under real-time ultrasound guidance; or video-assisted thoracoscopic lung biopsy on pulmonary nodules, masses, or infiltrative lesions can be used for obtaining tissues for cytology/histopathology [27].

A variety of positive staining methods have been described to demonstrate the yeast cells in tissue or fluids, ranging from the nonspecific Papanicolaou or hematoxylin and eosin stains to the more specific fungal stains such as Calcofluor, which binds fungal chitin, or Gomori methenamine silver (GMS), which stains the fungal cell wall [2, 25] (Fig. 15.6). Several stains can identify the polysaccharide capsular material surrounding the yeasts. These stains can be especially useful in presumptively identifying *Cryptococcus* when the organism does not grow or cultures are not obtained. They include Mayer's mucicarmine, periodic acid-Schiff (PAS), and alcian blue stains [2]. The Fontana-Masson stain appears to identify melanin in the yeast cell wall. The fungus is observed as a yeast that reproduces by formation of narrow-based budding with a prominent capsule. Gram stain is not optimal for identification of this yeast, but may show
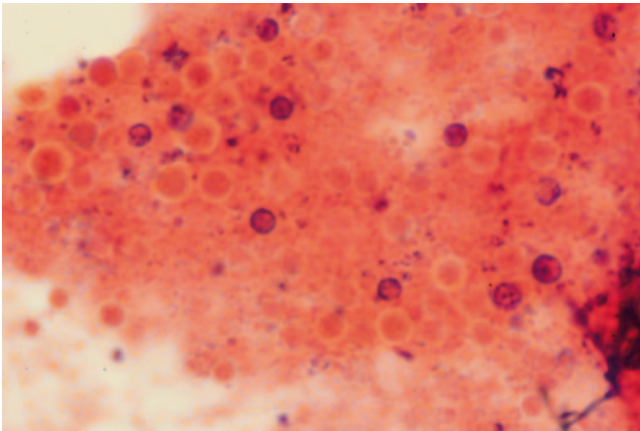
Fig. 15.7 Gram stain of sputum of a patient with pulmonary cryptococcosis. *Cryptococcus neoformans* appears as poorly stained gram-positive budding yeasts. (Courtesy of Dr. W. A. Schell, Duke University Medical Center)

C. neoformans as a poorly stained gram-positive budding yeast (Fig. 15.7) [2]. The recognition of *C. neoformans* in gram-stained smears of purulent exudates may be hampered by the presence of the large gelatinous capsule that apparently prevents definitive staining of the yeast-like cells.

Serology

Diagnosis of cryptococcosis has improved significantly over the past several decades with the development of serological tests for cryptococcal polysaccharide antigen and/or antibody. Use of serum cryptococcal antibodies for diagnosis of cryptococcosis has not been adopted. In contrast, detection of cryptococcal capsular polysaccharide antigen in serum or body fluids by a latex agglutination (LA) technique has been robust in its performance and is considered the gold standard diagnostic test for serological diagnosis of cryptococcosis. This test uses latex particles coated with polyclonal cryptococcal capsular antibodies or anti-GXM monoclonal antibodies and has overall sensitivities and specificities of 93–100 % and 93–98 %, respectively [28, 29]. The false-positive rate of cryptococcal capsular polysaccharide antigen testing is 0–0.4 % [30]. The majority of false-positive results can be explained by technical error (improper boiling/treatment), presence of rheumatoid factor or interference proteins, and infections with *Trichosporon beigelii* [31] or some bacterial species [32]. However, most of the false-positive results of LA testing for cryptococcal polysaccharide antigen have initial reciprocal titers of 8 or less [28]. Therefore, results of such low titers must be carefully interpreted within the clinical context. False-negative results of the LA test for cryptococcal polysaccharide antigen in cryptococcal meningitis are unusual but can

be seen due to a prozone effect, and, therefore, high-risk negative specimens should be diluted and retested [33]. Low fungal burden, as in chronic low-grade cryptococcal meningitis or in the very early stages of cryptococcal infection, and improper storage of patient sera can also cause false-negative results in LA cryptococcal polysaccharide antigen tests [34].

Enzyme immunoassays (EIAs) for detection and quantification of cryptococcal polysaccharide antigen of all four serotypes of *C. neoformans* in sera and CSF have been developed to detect the major component of the polysaccharide capsule, GXM, with sensitivities and specificities of 85.2–99 and 97 %, respectively [28, 35]. This methodology is automated and overcomes some of the practical limitations of LA testing. Previous studies have compared EIA and LA assays and revealed no significant difference between these testing methods. EIA for cryptococcal polysaccharide antigen does not give discrepant results with rheumatoid factor or serum macroglobulins and is not affected by prozone reactions. Both LA and EIA testing have been rigorously studied and are recommended for use in both serum and CSF samples.

Recently, a lateral flow assay (LFA) was introduced in the diagnostic repertoire for cryptococcal infection and is Food and Drug Administration (FDA) approved for use in serum and CSF. The semiquantitative LFA offers many advantages over other serological methods, including rapid turnaround (approximately 15 minutes), minimal requirements for specialized laboratory infrastructure, stability at room temperature, and low cost [36]. The LFA has been evaluated against both EIA and culture, with sensitivities of 96–100 % for serum and plasma and 70–94 % for urine samples [36–39]. This assay has good performance across a broad range of clinical settings, including resource-limited settings and among cohorts with low burden of HIV infection and high rates of *C. gattii* infection, for which some EIA and LA tests are known to be insensitive [36–40]. The satisfactory performance of LFA combined with established cost-effectiveness and practical advantages of this approach support its use as a point-of-care testing (including preemptive screening of high-risk patients) in resource-limited settings [36, 37, 41].

Although the presence of cryptococcal polysaccharide antigen in serum is undoubtedly suggestive for dissemination of cryptococcal infection outside the lung, the precise value of cryptococcal polysaccharide antigen for diagnosis of nondisseminated pulmonary cryptococcosis remains less certain. Generally speaking, detectable cryptococcal antigen in serum should make clinicians consider that infection is now also located outside the lung. In a high-risk patient with clinical symptoms suggestive of meningitis, identification of cryptococcal antigen in CSF or serum is rapid, specific, noninvasive, and virtually diagnostic of meningoencephali-

tis or disseminated cryptococcosis even when the India ink examination or culture is negative [42, 43]. The LA test for serum cryptococcal polysaccharide antigen is widely used for detecting cryptococcal infection in patients with AIDS, as an initial screening test for those with fever of unclear etiologies or neurological symptoms. In some patients, it may represent the only means of achieving an etiologic diagnosis of invasive cryptococcosis or early diagnosis prior to CNS involvement.

Likely because of its sensitivity, the detection of cryptococcal polysaccharide antigen in the serum may precede clinically obvious disseminated cryptococcal disease ("isolated cryptococcal polysaccharidemia") in severely immunosuppressed patients [44–46]. The management of these cases, in which there is a positive serum antigen and other nonspecific clinical findings in HIV-infected patients with negative fluid or tissue cultures, is uncertain. Persons of high risk with isolated cryptococcal antigenemia probably do benefit from antifungal therapy to prevent or delay the development of overt cryptococcosis [44]. Generally, positive serum antigen tests at titers of 1:4 or more strongly suggest cryptococcal infections in these patients.

Baseline cryptococcal polysaccharide antigen titers in serum and CSF may carry prognostic significance in patients with cryptococcal meningitis [47]. A study in HIV-related acute cryptococcal meningitis indicated that a baseline titer of CSF cryptococcal polysaccharide antigen of 1:1024 or greater was a predictor of death during systemic antifungal treatment [48]. After initiation of systemic antifungal therapy, patients may respond to treatment and titers of cryptococcal polysaccharide antigen fall. Similarly, a rise in CSF cryptococcal polysaccharide antigen titers during suppressive therapy has been associated with relapse [49]. However, it is important to emphasize that the use of changing antigen titers to make therapeutic decision should be done with caution, as titers may not be equivalent across different serological modalities [39]. The kinetics of polysaccharide elimination remains unclear and, despite the accuracy of commercial kits for general diagnosis, the accuracy of specific titers can vary from kit to kit even from the same clinical specimen.

Culture and Identification

Cryptococcus can be easily grown from biologic samples such as CSF, sputum, and skin biopsy on routine fungal and bacterial culture media. Colonies can usually be observed on solid agar plates after 48–72 h of incubation at 30–35 °C in aerobic conditions. Antibacterial agents, preferably chloramphenicol, can be added to the media when bacterial contamination is considered. The yeast, however, do not grow in the presence of cycloheximide at the con-

centration used in selective fungal isolation media (25 µg/mL). Despite relatively rapid growth for most strains, cultures should be held for 3–4 weeks before discarding, particularly for patients already receiving antifungal treatment. Conversely, cultures may be negative despite positive microscopic examinations (India ink) due to nonviable yeast cells, which may persist for a prolonged period of time at the site of infection. Positive blood cultures are frequently reported in AIDS patients and may actually be the first positive test for cryptococcal infection in a febrile high-risk patient.

C. neoformans colonies will appear on routine fungal media as opaque, white, creamy 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. *Cryptococcus* does not routinely produce hyphae or pseudohyphae, or ferment sugars, but is able to assimilate inositol and hydrolyze urea [50]. *C. neoformans* and *C. gattii* have the ability to use galactose, maltose, galactitol, and sucrose [50]. There are special media such as canavanine-glycine-bromthymol blue (CGB) agar that can be used to differentiate *C. gattii* strains from *C. neoformans* strains [51].

Molecular Identification Methods

A number of molecular techniques have been developed for identification of cryptococcal species from biological specimens including single and multiplex polymerase chain reaction (PCR) fingerprinting, random amplified polymorphic DNA (RAPD), PCR restriction fragment length polymorphism (RFLP) analysis, multi-locus sequence typing (MLST), and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry [52–58]. These highly sensitive and specific methods have been evaluated with a variety of biologic samples [59] and can rapidly identify to the species and subspecies/genotypic level, including identification of recognized and novel strains within geographical niches [60]. While the expense and specialized techniques required of these methods preclude widespread use in clinical practice, their use in larger-scale investigations will continue to enhance our understanding of the epidemiology, pathogenesis, and nuances of antifungal management, as well as identify microevolution of different strains [61].

Treatment

The Infectious Diseases Society of America Clinical Practice Guidelines for the Management of Cryptococcal Disease (summarized in Tables 15.5, 15.6), updated in 2010,

Table 15.5 Treatment recommendations for cryptococcal meningoencephalitis. (Adapted from the 2010 IDSA Clinical Practice Guideline for the Management of Cryptococcal Disease with personal suggestions [62])

Human immunodeficiency virus-infected individuals[a]	
Induction therapy:	
Primary regimen	
AmBd (0.7–1 mg/kg/day) plus flucytosine (5-FC; 100 mg/kg/day)	2 weeks
Liposomal AmB (3–4 mg/kg/day) or AmB lipid complex (ABLC; 5 mg/kg/day)	
pls 5-FC(100 mg/kg/day) for patients predisposed to renal dysfunction	2 weeks
Alternative regimens[b]	4–6 weeks
AmBd (0.7–1 mg/kg/day) or liposomal AmB[c] (3–4 mg/kg/day) or ABLC (5 mg/kg/day) if flucytosine-intolerant	2 weeks
AmBd (0.7–1 mg/kg/day) plus fluconazole (800 mg/day)	6 weeks
Fluconazole (>800 mg/day, preferably 1200 mg/day) plus 5-FC (100 mg/kg/day)	10–12 weeks
Fluconazole (800–2000 mg/day, preferably 1200 mg/day)	10–12 weeks
Itraconazole (200 mg BID)	8 weeks
Consolidation therapy: fluconazole (400 mg/day)	>1 year[d]
Maintenance or suppressive therapy: fluconazole (200 mg/day)	>1 year[d]
Alternative regimens[b]	
Itraconazole (200 mg BID)	>1 year[d]
AmBd (1 mg/kg IV per week)	
Organ Transplant Recipients[e]	
Induction therapy:	2 weeks
Primary regimen	
Liposomal AmB (3–4 mg/kg/day) or ABLC (5 mg/kg/day) plus 5-FC (100 mg/kg/day)	4–6 weeks
Alternative regimen (if flucytosine-intolerant)	
Liposomal AmB[c] (up to 6 mg/kg/day) or ABLC (5 mg/kg/day)	4–6 weeks
AmBd (0.7 mg/kg/day)[g]	
Consolidation therapy: fluconazole (400–800 mg/day)	8 weeks
Maintenance or suppressive therapy: Fluconazole (200–400 mg/day)	6–12 months
Non-HIV-infected and nontransplant patients	
Induction therapy:	
Primary regimen	
AmBd (0.7–1 mg/kg/day) plus 5-FC (100 mg/kg/day)	4–6 weeks[f]
Alternative regimens	
Liposomal AmB (3–4 mg/kg/day) or ABLC (5 mg/kg/day) plus 5-FC (100 mg/kg/day)	4 weeks
AmBd (0.7–1 mg/kg/day)	6 weeks
Liposomal AmB (3–4 mg/kg/day) or ABLC (5 mg/kg/day)	6 weeks
Consolidation therapy: fluconazole (400–800 mg/day)	8 weeks
Maintenance therapy: fluconazole (200 mg/day)	6–12 months

ABLC AmB lipid complex, *AmB* amphotericin B, *5-FC* flucytosine

[a] Initiate HAART 2–10 weeks after beginning antifungal regimen. Shorter duration (i.e., 2 weeks) of induction therapy can be considered for certain low-risk patients

[b] Can be considered as alternative regimen in circumstances in which primary regimen not available but are not encouraged as equivalent substitutes

[c] Liposomal amphotericin can be safely administered in doses as high as 6 mg/kg/day

[d] After 1 year of therapy, if successful response to ARVs (CD4 count>100 and viral load low or undetectable for>3 months), discontinuation of antifungal therapy can be considered. Consider reinstitution if CD4 count falls below 100

[e] Consider stepwise de-escalation of immunosuppressive regimen if allograft function permits

[f] If CSF culture remains positive at 2 weeks of therapy or initial presentation with neurologic complications, longer therapy preferred

[g] Caution due to concomitant calcineurin inhibitor use

provide a suitable framework for therapeutic decision making [62]. The updated guidelines provide detailed recommendations for specific "at-risk" populations and address different management strategies based on host, site of infection, and potential complications of cryptococcal infection. While subtle nuances exist based on host and site of infection, general principles for the management of cryptococcal infection can provide the cornerstone of a treatment plan in most cases.

Table 15.6 Treatment recommendations for nonmeningeal cryptococcosis

Immunosuppressed[a] and immunocompetent,[b] mild-to-moderate pulmonary disease Fluconazole (400 mg/day) *Alternatives (immunocompetent):* itraconazole 200 mg BID, Voriconazole 200 mg BID or posaconazole 400 mg BID	6–12 months[c]
Immunosuppressed or immunocompetent, severe pulmonary disease Treat as CNS disease	12 months
Nonmeningeal, nonpulmonary cryptococcosis *Patients with cryptococcemia* Treat as CNS disease	12 months
No CNS disease, no fungemia, isolated focus of infection Fluconazole 400 mg/day	6–12 months

CNS central nervous system

[a] CSF sampling should be performed to rule out CNS involvement

[b] CSF sampling can be considered but not required in the absence of neurological symptoms or high serum cryptococcal antigen

[c] If successful response to ARVs (CD4 count > 100 and viral load low or undetectable for > 3 months) and stable serum cryptococcal antigen, can consider discontinuation of antifungal therapy after 12 months of therapy

Basic Management Principles/Role of Combination Therapy

Amphotericin B deoxycholate (AmBd) remains the foundation of treatment for disseminated cryptococcosis and severe cryptococcal infection. A standard induction dose of 0.7–1 mg/kg/day is recommended. Liposomal amphotericin B (AmBisome) at 3–6 mg/kg/day has become a preferred alternative treatment with similar outcomes to that of AmBd but with less nephrotoxicity and is specifically recommended for primary induction in organ transplant patients as well as patients at risk for renal dysfunction [62–64]. Higher doses of AmBd have been shown to be more rapidly fungicidal [65, 66]. Flucytosine (5-FC) is primarily used in combination therapy with AmBd for first-line therapy in cryptococcal meningitis or severe pulmonary cryptococcosis at a dosage of 100 mg/kg/day in divided doses in patients with normal renal function [67, 68]. The combination of AmBd and 5-FC represents the most potent fungicidal regimen with more rapid sterilization of CSF cultures at 2 weeks as demonstrated by multiple studies [66, 67, 69]. Early studies from HIV infection demonstrated increased rates of CSF sterilization and fewer relapses with the combination of AmBd and 5-FC followed by itraconazole maintenance [67]. This initial combination regimen has since been compared against multiple alternatives, with the superiority of its fungicidal activity consis-

tently confirmed [69]. Similar results have been observed among the most severe cases of cryptococcal infection [66, 70]. Early mycological failure (as defined by persistently positive CSF cultures at day 14) has for many years been associated with late treatment failure and poor outcome [71], and lack of 5-FC has been independently associated with both early [72] and late [70] mycological failure. The improved fungicidal activity of combination therapy with AmBd plus 5-FC has been shown to translate into a direct survival benefit compared with AmBd monotherapy, with improved survival at 10 weeks lasting up to 6 months [66]. 5-FC should be dose-adjusted for renal dysfunction, with therapeutic monitoring performed 3–5 days after initiation of therapy, to maintain 2-h post-dose levels under 100 μg/mL (goal 30–80), to reduce its primary side effect of bone marrow suppression [73].

Alternative Combination Regimens

Though combination induction therapy with AmBd and 5-FC remains the recommended standard of care for severe cryptococcosis including cryptococcal meningitis, limited availability of 5-FC in resource-limited settings presents significant challenges for managing patients in areas where the disease burden and mortality rates are highest. Alternative combination therapies have been investigated, the most efficacious of which has been AmBd (0.7 mg/kg/day) plus fluconazole (800 mg/day), which has demonstrated improved rates of a composite end point of CSF culture negativity, neurological improvement, and survival compared with AmBd alone or in combination with lower doses of fluconazole [74]. Fluconazole (at doses of 800–1200 mg/day) in combination with AmBd (standard dosing) has been shown to demonstrate similar rates of fungal clearance from CSF as standard AmBd plus 5-FC in a randomized study performed in HIV-infected patients in South Africa [75] and offers a potential viable option for effective initial therapy in settings where access to 5-FC is limited. Whether the survival benefit observed with AmBd plus 5-FC will be observed with this regimen remains uncertain. Additional alternative regimens for primary therapy are available in the guidelines but their use is not encouraged based on limited data on the success of these regimens [76]. Use of fluconazole in the absence of a polyene is not recommended given the fungistatic nature of this drug, poor success, higher relapse rates, and increased resistance in relapse when used as monotherapy for induction [62, 77]. However, in areas without access to AmBd, high doses (1200 mg/day) of fluconazole should be commenced.

Host Considerations

Cryptococcal Meningitis in HIV Patients

A three-stage regimen of induction/consolidation/maintenance is employed in the treatment of cryptococcal meningitis in all patients, irrespective of host risk factors [62, 67]. In HIV-infected patients, the initial induction treatment usually begins with combination therapy with AmBd plus 5-FC for at least 2 weeks as above, followed by consolidation treatment with fluconazole 400–800 mg/day for 8 weeks in patients who have demonstrated favorable response. Following consolidation, a long-term suppressive/maintenance phase is commenced with oral fluconazole, 200–400 mg given once daily. This has been demonstrated to effectively reduce rates of relapse from ~40% to less than 5% in the pre-HAART era [78]. Secondary prophylaxis can be discontinued after 1–2 years of antifungal therapy in patients who respond to HAART with rise in CD4$^+$ cell counts to greater than 100 cells/µl and decline in viral load (HIV RNA) to undetectable levels for at least 3 months [62, 79, 80].

Itraconazole can be used as an alternative consolidation treatment for cryptococcosis, but first-line therapy is with fluconazole. Despite its poor CSF penetration and inconsistent oral bioavailability, itraconazole has been successfully used in the treatment of cryptococcal meningitis [81]; however, it has been shown to be inferior to fluconazole during the suppression phase [82] and requires therapeutic drug monitoring due to its poor bioavailability. Newer triazoles including posaconazole and voriconazole are not specifically incorporated into practice guidelines but are active against cryptococcal isolates in vitro and have been shown to demonstrate moderate efficacy in patients with refractory disease [83, 84].

In patients with HIV-associated cryptococcal infection, HAART has a major impact on long-term prognosis. However, given concerns regarding immune reconstitution inflammatory syndrome (IRIS), the optimum timing for HAART initiation in the setting of OIs has been a subject of much debate. Early retrospective studies suggested an increased risk of IRIS among HIV-infected patients initiated on HAART early after the diagnosis of an OI [85, 86]. More contemporary studies have demonstrated conflicting results regarding outcomes of cryptococcal infection based on timing of HAART initiation [87–91]. The Cryptococcal Optimal ART Timing (COAT) study provides the best evidence for current recommendations regarding timing of HAART initiation in patients with cryptococcal meningitis [92]. HAART-naïve patients were randomized to receive immediate (within 48 h) or deferred (greater than four weeks) HAART following a minimum of 7 days of antifungal therapy with AmBd and high-dose fluconazole. This trial was stopped early after interim analyses suggested poorer early survival among patients receiving immediate HAART (55% vs. 70%, $p=0.03$), particularly among patients with altered mentation and low CSF white blood cell count. Although a trend toward increased rates of and earlier IRIS was observed in the immediate HAART group, this was not statistically significant.

The above data support recommendations to delay initiation of HAART in patients with cryptococcal meningitis for a minimum of 4 weeks after starting antifungal therapy (potentially longer if the primary regimen does not include AmBd) and after demonstration of a sustained clinical response to antifungal therapy [62, 93]. Interruption of HAART and/or corticosteroid treatment may be used to control symptoms if severe cryptococcal IRIS occurs.

Organ Transplant Recipients

Organ transplant recipients with CNS cryptococcal infection are managed similar to HIV-infected patients, with the exception of preferential use of lipid formulations of amphotericin B to limit nephrotoxicity [62]. The principles of induction, consolidation, and maintenance therapy remain the same. Repeat CSF sampling at 2 weeks is recommended in this population and a longer course of induction therapy should be pursued if CSF cultures remain positive at 2 weeks, as this scenario is associated with increased 6-month mortality [94]. Unlike HIV-infected patients, relapse rates among organ transplant recipients are quite low (~1.3%), such that a shorter course of maintenance therapy with fluconazole (6–12 months) can be pursued following standard consolidation [62, 94]. Drug interactions between fluconazole and immunosuppressive agents should be anticipated due to fluconazole-induced CYP3A4 inhibition, and preemptive adjustment (reduction) in calcineurin inhibitors should be made. Management of immunosuppression in the setting of cryptococcal infection requires recognition of the increased risk of IRIS associated with abrupt withdrawal or reduction of immunosuppression in organ transplant recipients with increased rates of allograft loss reported in some patients [95–97]. Stepwise reduction in immunosuppression is recommended, though the approach should be individualized for each patient.

Non-HIV-Infected, Nontransplant Patients

Screening for HIV and CD4 lymphopenia is recommended among patients who present with cryptococcosis without apparent risk factors [62]. Very little prospective data are available on the management of cryptococcal infection among this heterogeneous group of "apparently immunocompetent" patients lacking classical risk factors for cryptococcosis. What is known is based on early studies that included a heterogeneous mix of patients and were performed prior to acceptance of the standard algorithm of induction, consolidation, and maintenance therapy and higher-dose polyene

therapy [68]. Recommendations for longer induction therapy (4 weeks or more) in this population are based on the recognition of poorer outcomes and higher mortality rates in this group of patients in both early [68, 98] and contemporary [99] studies. An additional 2 weeks of therapy should be considered if 5-FC is not included in the induction regimen [62]. Recommendations for consolidation and maintenance parallel those for HIV-infected and transplant patients, and reflect early reports of relapse rates approaching 30 % within the 1st year prior to introduction of consolidation and maintenance antifungal therapy [62, 68]. Criteria for stopping treatment in these patients include resolution of symptoms, generally following at least 1 year of suppressive therapy. Patients may have prolonged positive cryptococcal polysaccharide antigen tests and/or slightly abnormal CSF findings for months during successful therapy, and if there are concerns about cure, follow-up CSF culture should be considered.

Nonmeningeal Cryptococcosis

Just as host factors influence management approaches for cryptococcal infection, site of infection also matters. Airway colonization in a nonimmunosuppressed individual poses a low risk for invasive pulmonary infection (and dissemination) and treatment can be deferred. Some experts would still favor treatment with fluconazole in this scenario, given the relative benign nature of this therapy. However, among immunocompromised patients with isolated pulmonary cryptococcosis, treatment is recommended to prevent dissemination [62]. It should be emphasized that a thorough evaluation to rule out systemic disease/dissemination is warranted in this group of patients to provide optimal treatment. This includes blood and CSF cultures as well as serum and CSF cryptococcal antigen testing. If the results of the above evaluation are negative, symptoms are mild, and there is no evidence of diffuse pulmonary infiltrates or ARDS, oral fluconazole (400 mg/day) is recommended for 6–12 months. However, in any patient in whom cryptococcemia is identified, symptoms are severe, ARDS is present, or CSF examination reveals asymptomatic CNS involvement, treatment for cryptococcal meningitis is recommended [62]. Cerebral cryptococcomas often can be managed with prolonged antifungal therapy without need for surgical removal unless mass effect or other evidence of obstruction is identified. At least 6 weeks of induction therapy with AmBd plus 5-FC, followed by 6–18 months of consolidation therapy with fluconazole (400–800 mg/day), is recommended for management. Surgery should be considered for large lesions (>3 cm) or the presence of obstructive hydrocephalus [62]. Localized infection of extrapulmonary nonmeningeal sites can occasionally occur 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, as management strategies will differ if disseminated disease is present. Consultation with ophthalmology is indicated in cases of cryptococcal eye disease [62].

Immune Reconstitution Inflammatory Syndrome

Restoration of pathogen-specific immunity as a result of HAART or following reduction of immunosuppression in SOT recipients can result in a destructive inflammatory response known as the *immune reconstitution inflammatory syndrome* (IRIS). IRIS is best characterized in association with *C. neoformans* infection of the CNS, particularly among HIV-infected patients, and is associated with significant morbidity and mortality [85, 86, 88, 89, 100–108]. Proposed criteria for IRIS include onset of symptoms within 12 months of HAART initiation (with concomitant CD4+ recovery) [109]. In addition, IRIS is estimated to occur in 5–11 % of SOT recipients with cryptococcal infection and has been associated with an increased risk of allograft failure [95, 110–114]; cryptococcal IRIS may also be observed in non-HIV-infected, nontransplant patients [115].

Clinical features of cryptococcal IRIS are similar to cryptococcal infection, most commonly presenting as CNS disease, although lymphadenitis, pneumonitis, multifocal disease, soft-tissue involvement, and mediastinitis have all been reported [109, 116]. Meningeal disease is the most frequent and most serious presentation [109]; aseptic meningitis with associated intracranial hypertension and CSF pleocytosis is most commonly observed [100, 102, 103, 105, 106, 108]. A hallmark histopathologic finding is suppurative or necrotic granulomatous inflammation with yeast seen in tissues despite negative tissue cultures [95, 112, 116, 117]. The presence of a positive CSF culture in cases of suspected cryptococcal IRIS should raise suspicion for direct antifungal failure or resistance, particularly in settings where fluconazole therapy is widely used as the standard of care [88].

Cryptococcal IRIS represents unchecked reversal of a Th2 (anti-inflammatory) to Th1 (pro-inflammatory) immune response in the setting of immune reconstitution [118]. Prospective cohort studies of HAART-naïve individuals indicate that an ineffectual host immune response to initial infection is associated with a greater likelihood of future IRIS [105]. A three-phase theory of cryptococcal immune reconstitution has been postulated, marked by: (1) failure of antigen clearance due to inappropriate Th2 response; (2) lack of effector response despite inflammatory signaling; and, ultimately, (3) vigorous pro-inflammatory responses (both Th1 and Th17) to residual antigen, recognized clinically as IRIS [100].

There are no reliable diagnostic tests for IRIS, and establishing the diagnosis presents a considerable challenge [101, 119]. The differential diagnosis includes progressive

disease due to persistent immune deficiency, failure of antimicrobial therapy (due to resistance or nonadherence), coinfection with other OIs, and drug toxicity. A high index of suspicion is necessary for recognizing atypical presentations or manifestations at distant sites. Nevertheless, distinguishing between disease progression related to ongoing immune deficiency and clinical deterioration due to restoration of host immunity has important management implications. CSF analyses and biomarkers may be useful in distinguishing between relapse and IRIS. Prospective studies have demonstrated that CSF opening pressure [89] and WBC count [100, 105] at the time of an IRIS event are significantly higher than baseline values for individual patients, and higher CSF opening pressures can distinguish IRIS from relapsed infection [102].

Treatment options for cryptococcal IRIS are based largely on expert opinion [62]. Implicit in management is ensuring the efficacy of antifungal therapy, particularly in settings where access to AmBd may be limited and fluconazole resistance may account for recurrent meningitis episodes [120, 121]. In the absence of disease relapse or direct antifungal resistance, modification of antimicrobial therapy is not indicated [62]. Once the diagnosis of IRIS is suspected, consideration of disease severity is warranted. A significant proportion of minor cases will improve without specific treatment [86, 88, 108]. Corticosteroids have been shown to reduce the need for hospitalization and to improve short-term quality of life and functional status without increased risk of complications in paradoxical tuberculosis (TB)-associated IRIS [122]; the role of corticosteroids in cryptococcal IRIS, however, is not as well established and should be reserved for life-threatening cases, particularly in light of their association with increased mortality in one study [123]. Other anti-inflammatory agents have been used in cryptococcal IRIS, but the number of patients treated with any of these agents is too small to draw substantive conclusions [86, 124, 125]. Other management strategies, including therapeutic lumbar drainage in the setting of intracranial hypertension [62, 122, 126] and, at times, surgical drainage of suppurative lymph nodes [116, 117], are important adjunctive therapies that may be considered in severe disease.

Although no controlled studies have been performed, continuation of HAART in the setting of IRIS is recommended and has been performed safely without adverse effects in several studies [87, 88, 103, 119, 127]. Similarly, withdrawal or reduction of immunosuppressive agents is standard practice in managing infectious complications in SOT recipients [111]. Given the putative risk of IRIS with abrupt withdrawal or discontinuation of immunosuppressive agents in these patients, gradual de-escalation during the initiation of antifungal therapy is advised to reduce the risk of future IRIS [95, 111, 112].

Persistent and Relapsed Infection

Persistent and relapsed infection must be distinguished from IRIS, as management strategies will differ significantly. Persistent disease can be defined as persistently positive CSF cultures after 1 month of antifungal therapy, whereas relapse requires new clinical signs and symptoms and repeat positive cultures (at same or distant sites) after initial improvement and fungal sterilization [62]. 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 cases include resumption of induction therapy, often for a longer duration and at increased dosages, if tolerable, and pursuance of antifungal susceptibility testing [62].

Antifungal Susceptibility Testing

While routine in vitro susceptibility testing of cryptococcal isolates at the time of initial therapy is not recommended, there is a role for testing in cases of suspected relapse or persistent infection [62]. It is generally recognized that primary antifungal resistance to most agents is rare, although reduced susceptibility to 5-FC has been observed in untreated patients [128] and echinocandins have no reliable activity against this yeast. Reduced susceptibility to fluconazole has been described in cases of culture-positive relapsed cryptococcal meningitis associated with prior fluconazole therapy [77, 129, 130] (Table 15.6).

Management of Elevated CSF Pressure

Along with the optimization of antifungal therapy, management of increased ICP is critically important. Elevated ICP is correlated with overall fungal burden, and is thought to be due to CSF outflow obstruction by clumped yeast forms [131]. An ICP of 250 mm H_2O or greater is considered elevated and is associated with increased morbidity and mortality [123]. Persistently elevated ICP after 2 weeks of treatment is associated with poorer clinical responses among patients with HIV-associated cryptococcal meningitis [62]. Intracranial imaging should be performed prior to lumbar puncture if impaired mentation or focal neurologic deficits are present. A baseline measurement of CSF pressure should be obtained in all patients with suspected cryptococcal meningitis. Aggressive attempts to control increased ICP should occur, if elevated and if there are signs/symptoms to suggest increased ICP (headache, mental status changes, and new focal neurological findings). Treatment options for managing acutely elevated ICP include repeated lumbar punctures

Table 15.7 Management of elevated intracranial pressure in HIV-infected patients with cryptococcosis. (Based on the IDSA Practice Guideline for the Management of Cryptococcal Diseases [62])

| **Initial lumbar puncture** |
| Positive focal neurological signs or altered conscious status |
| Radiographic imaging before lumbar puncture is indicated |
| Normal opening pressure |
| Initiate medical therapy, with follow-up lumbar puncture at 2 weeks |
| Opening pressure ≥250 mm H_2O with signs or symptoms |
| Lumbar drainage sufficient to achieve closing pressure |
| <200 mm H_2O |
| or 50% of initial opening pressure[a] |
| **Follow-up for elevated pressure** |
| Repeated drainage daily until opening pressure |
| <200 mm H_2O and symptoms/signs |
| are stable |
| If elevated pressure persists, consider |
| Lumbar drain |
| Ventriculoperitoneal shunt |

[a] Recommendations are not evidence based and are provided as a guide only

(daily until pressure and symptoms are stable for >2 days), lumbar drain insertion, ventriculostomy, or ventriculoperitoneal (VP) shunt (Table 15.7) [123]. Medical treatments such as corticosteroids (unless there is a component of IRIS), mannitol, and acetazolamide have been used in some cases, but are generally not recommended for use in management of increased ICP in cryptococcal meningitis [132]. Some patients may develop symptoms of obstructive hydrocephalus necessitating the placement of a permanent VP shunt during the first 1–2 years of treatment, and occasionally at initial presentation. Sterilization of CSF is not required prior to placement of a VP shunt, which can be inserted once a patient is receiving the appropriate antifungal therapy [133].

Prevention

Prevention of cryptococcal disease can be achieved by use of HAART in HIV-infected patients. Fluconazole prophylaxis has been shown to be effective for preventing cryptococcosis in AIDS patients with persistently low CD4$^+$ cell counts (below 100 cells/μl) [134, 135], but due to concerns regarding antifungal resistance, this approach is not currently recommended, and HAART remains the best strategy for the prevention of cryptococcal disease in this population. Routine screening for cryptococcal infection and/or prophylaxis are not recommended in SOT recipients, even when immunosuppression is augmented in patients with previously (appropriately) treated infection [136]. Although cryptococcal GXM–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 [137, 138].

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