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Review Article

Review Article

Pulmonary cryptococcosis: A review of pathobiology and clinical aspects

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Abstract

Pulmonary cryptococcosis is an important opportunistic invasive mycosis in immunocompromised patients, but it is also increasingly seen in immunocompetent patients. The main human pathogens are *Cryptococcus neoformans* and *C. gattii*, which have a worldwide distribution. In contrast to cryptococcal meningitis, pulmonary cryptococcosis is still underdiagnosed because of limitations in diagnostic tools. It can mimic lung cancer, pulmonary tuberculosis, bacterial pneumonia, and other pulmonary mycoses both clinically and radiologically. Pulmonary nodules are the most common radiological feature, but these are not specific to pulmonary cryptococcosis. The sensitivity of culture of respiratory samples for *Cryptococcus* is poor and a positive result may also reflect colonisation. Cryptococcal antigen (CrAg) with lateral flow device is a fast and sensitive test and widely used on serum and cerebrospinal fluid, but sera from patients with pulmonary cryptococcosis are rarely positive in the absence of disseminated disease. Detection of CrAg from respiratory specimens might assist the diagnosis of pulmonary cryptococcosis but there are very few data. Molecular detection techniques such as multiplex reverse transcription polymerase chain reaction (RT-PCR) could also provide better sensitivity but these still require validation for respiratory specimens. The first line of treatment for pulmonary cryptococcosis is fluconazole, or amphotericin B and flucytosine for those with central nervous system involvement. Pulmonary cryptococcosis worsens the prognosis of cryptococcal meningitis. In this review, we summarize the biological aspects of *Cryptococcus* and provide an update on the diagnosis and management of pulmonary cryptococcosis.

Key words: Cryptococcal pneumonia, cryptococcal infection of lungs, *Cryptococcus neoformans*, *Cryptococcus gattii*.

Introduction

Pulmonary cryptococcosis is commonly seen in immunocompromised patients and it has become an emerging disease in immunocompetent patients.[1–7] A study from Uganda reported that 11% of hospitalized patients with human immunodeficiency virus (HIV) infection had pulmonary cryptococcosis as a secondary infection.[8] Recent research from Thailand noted that 13% of HIV patients with pneumonia had cryptococcal antigen present in their serum, although a third of this population did not have cryptococcal meningitis or a history of it.[9] Most likely the respiratory infections in these patients was cryptococcal. A retrospective review from China revealed that 60% of pulmonary cryptococcosis cases were diagnosed in immunocompetent non-HIV patients.[10] Pulmonary cryptococcosis may also disseminate

Table 1. Classification and distribution of *Cryptococcus* spp.*

Species	Varieties	Serotypes	Subgroup by MLST	Most common niches	Distribution (more prevalent regions)
C. neoformans	var. *grubii*	A	VNI	Pigeons, other birds, trees, soil	Global
	var. *grubii*	A	VNII	Unknown	Global (Australia, Africa, North America)
	var. *grubii*	A	VNB	Trees (mopane)	Southern Africa (Botswana, South Africa)
	var. *neoformans*	D	VNIV	Pigeons, soil, other birds	Global (Europe)
	Hybrids	AD hybrid	VNIII	Pigeons, soil, other birds	Global (Europe)
C. gattii	–	B or C	VGI	Trees (eucalypt)	Global (Australia)
	–	B or C	VGII	Trees (eucalypt)	North America (Canada, United States), South America (Columbia)
	–	B or C	VGIII	Trees (almond)	South America
	–	B or C	VGIV	Trees	North America

*Adapted from Mitchell et al.[21] and Chang et al.[22]

and lead to fatal complications.[11–13] A previous study reported that 67% of pulmonary cryptococcosis in immunocompetent patients disseminated into the central nervous system causing cryptococcal meningitis.[11] Patients with pulmonary cryptococcosis concomitantly with cryptococcal meningitis showed inadequate treatment response and poor clinical outcome compare to cryptococcal meningitis alone.[14] The spectrum of fungal diseases seen in critically ill patients and immunocompetent patients is broadening and the role of pulmonary cryptococcosis may be increasingly important.[15–18] We elected to review this topic, with a focus on pulmonary cryptococcosis.

Methods

A literature search of all published articles in the English language within the last 50 years (1968–2018) was conducted using the PubMed database. Key search terms included 'pulmonary cryptococcosis', 'cryptococcal pneumonia', 'cryptococcal infection of lungs', '*Cryptococcus neoformans*', and '*Cryptococcus gattii*'. Additional references were obtained by a thorough manual scanning of the bibliographies listed in the selected articles. All relevant articles (n = 275) have been included into this review.

Cryptococcus

Cryptococcus is a genus of encapsulated yeasts belonging to the Basidiomycota phylum and is widely distributed around the world.[19–22] The most common environmental niches of *Cryptococcus* are lignaceous environments such as hollows in trees, flowering plants, and bird feces.[23–25] In addition, *Cryptococcus* has been found in arctic climates and under conditions of extremes of pH (Table 1).[21,22,26–29] Some *Cryptococcus* species are pathogenic to humans and some animals and typically cause meningitis and infections in the airways. The portal of entry is normally in the respiratory tract.[30] *Cryptococcus* infections have been reported in a broad range of animals, including cats, dogs, horses, birds, and koala bears.[31] Cryptococcal rhinosinusitis in dogs and cats, lower respiratory tract cryptococcosis in koala, and cryptococcal mastitis in cattle are examples of cryptococcosis in animals. In contrast, many birds are carriers of *Cryptococcus* and contribute to its spread but rarely become infected themselves.[21,32,33]

The genus *Cryptococcus* consists of more than 70 species that exhibit significant biodiversity between them.[34–36] The two main pathogenic cryptococcal species for humans and animals are *C. neoformans* and *C. gattii* that belong to the *C. neoformans* species complex.[37] Non- *C. neoformans species*, such as *C. laurentii, C. uniguttulatus*, and *C. albidus* may cause infections.[38] Other *Cryptococcus* spp. are less pathogenic probably because of a weaker capsular structure although can be fatal in humans as observed in *C. liquefaciens*.[39–42] *C. neoformans* is commonly found in pigeon droppings, whereas *C. gattii* is more likely to be found in vegetation, such as eucalyptus trees.[21,23] Predisposing factors that support the survival and the growth of *C. neoformans* in pigeon droppings are an acidic pH, a high concentration of uric acid and glucose, the ability of *C. neoformans* to survive against desiccation, and minimal competition with bacterial species compared to other animal excreta.[43–45] The worldwide migration of many bird species supports the ubiquitous spread of *C. neoformans* whereas *C. gattii* is confined to endemic pockets.[21,46]

Serotype grouping is based on capsular polysaccharide agglutination patterns, and the two main species of *Cryptococcus* (*C. neoformans* and *C. gattii)* have been serotyped as A, B, C, and D.[35,47] Each serotype or their combination represents a specific variety within *C. neoformans* species (Table. 1).[22,35,48,49] Common varieties of *C. neoformans* are *grubii* (serotype A) and *neoformans* (serotype D). In contrast, *C. gattii* serotypes are not classified into varieties and they all belong to serotypes B or C.[48,50,51] One shortfall of the serotype classification system is

antigenic evolution.[52] For example, the CBS 132T type strain of *C. neoformans* was initially identified as serotype D but more recently has been found as an AD hybrid.[53] *C. neoformans* var. *grubii* is estimated to be the pathogen in 90% of cryptococcosis cases worldwide and causes more severe infection than other species.[36,54–56]

Genetic diversity of *Cryptococcus*

Molecular genotyping methods have revealed a wide genetic diversity of *Cryptococcus*.[57] There are wide genotype and phenotype divergences between *C. neoformans* and *C. gattii*.[53] The main methods used have been DNA and polymerase chain reaction (PCR) fingerprinting, multi-locus sequence typing (MLST), amplified fragment length polymorphism (AFLP) analysis, and multigene and intergenic spacer (IGS) sequences of the ribosomal DNA analysis.[58–62] Interlaboratory reproducibility using PCR fingerprinting and AFLP analysis is poor. MLST types have been shown to associate more closely with the virulence and patterns of infection than serotyping in both immunocompetent and immunocompromised hosts.[63–66] Therefore, the Cryptococcal Working Group 1 (genotyping of *C. neoformans* and *C. gattii*) of the International Society for Human and Animal Mycology (ISHAM) has recommended MLST as the primary method for cryptococcal strain typing.[57] This working group also promotes the use of VNI-VNIV and VGI-VGIV nomenclature for *Cryptococcus* isolates[57,67] (Table 1).

Genetic variations are linked to biochemical and physiological differences including serotypes, the profile of matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS), pathophysiology, and clinical manifestations.[59,68–71] Using phylogenetic analysis on IGSI, IGSII, and IGSI + 5S ribosomal ribonucleic acid (rRNA) + IGSII regions of *Cryptococcus* found at least six genotypes of *Cryptococcus,* each of them with various subgroups.[59] In recent years, there has been increasing interest in proposing *C. neoformans* var. *neoformans* (serotype D) as a different species from *C. neoformans* var. *grubii* (serotype A).[36,53] Similarly, another suggestion is to divide *C. gattii* into five different species because of their apparent differences of phylogenetic analysis and population structure.[53] In contrast, some scientists prefer to apply "*C. neoformans* species complex and "*C. gattii* species complex," as commonly used for other fungi and mycobacteria.[72] However, no consensus has yet been reached on nomenclature.

Global epidemiology of *Cryptococcus*

C. neoformans and *C. gattii* have a worldwide distribution with their specific areas for different serotypes[32] (Table 1). In the ARTEMIS DISK global antifungal surveillance project (1999–2007) *C. neoformans* was the most common yeast isolated after *Candida*.[73,74] Of the clinical non-*Candida* yeast isolates, 31% were identified as *C. neoformans,* and 1% as *C. gattii*.[73] However, *C. gattii* is an emerging pathogen responsible, for example, for an ongoing epidemic in North America and Vancouver Island in immunocompetent patients.[75,76] There is also evidence of its spread outside the original endemic area.[77–79] A recent epidemiological study covering Asia, Africa, America, Europe, and Oceania and analyses of 68,811 isolates of *C. neoformans* and *C. gattii* revealed that *C. neoformans* var. *grubii* molecular type VN1 is the most common type except in Oceania.[80–83] There (Australia, New Zealand, Papua New Guinea, and Hawaii), *C. gattii* type VG1 was the most common types representing 39% of the total isolates, consistent with the endemicity of *C. gattii*.[80] These conclusions were based on a combination of clinical and environmental isolates, with a dominance of clinical isolates (83%). Another study showed similarly that *C. neoformans* var. *grubii* serotype A represents 81% of all *Cryptococcus* isolated from all over the world.[84]

Different levels of genetic diversity have been reported in different countries in Asia.[66,85] Those of Southeast Asia (Indonesia, India, and Thailand) appear to have more cryptococcal polymorphisms than those of East Asia (China, Hong Kong, Japan). A rare type (ST93) has been reported to be predominant in Indonesian and Indian isolates.[66,85]

Pathogenesis of pulmonary cryptococcosis

Cryptococcus and the host response

The most common means of *Cryptococcus* causing infection in humans is via inhalation.[86–88] Spores and small desiccated yeast cells (approximately 1–5 μm in diameter) of *Cryptococcus* may reach the lower airways and pulmonary alveoli.[89] Respiratory defences such as mucociliary transport, airflow turbulence, physical epithelial mucosal barrier, and mucus trapping do not prevent these small infectious propagules reaching the distal lung.[89–92]

Both innate and adaptive immune systems are involved in host response against *Cryptococcus* infection.[19,93] Innate immune responses including lung surfactant, complement, dendritic cells (DCs), eosinophils, and alveolar macrophages (AMs) are activated in the initial step of *Cryptococcus* infection.[93] DCs, AMs, and eosinophils have a role as phagocytic cells against *Cryptococcus*.[93–95] Lung surfactant contains SP-D protein that likely binds with *Cryptococcus* and attracts eosinophils.[96]

In alveolar spaces, AMs phagocytose *Cryptococcus*.[97,98] A granulomatous immune response may follow with the formation of a subpleural nodule or primary lymph node complex.[99,100] Murine models support this sequence of events, as *C. neoformans* infection results in hilar lymph node infection.[101] Pulmonary nodules are a common manifestation on radiologic examination.[102]

Finally, DCs also stimulate T lymphocytes (T cells) as the key adaptive immune response. CD4+ T cells have a central

role in the defence against cryptococcal infection. CD4+, CD8+ and natural killer (NK) cells act as fungistatic agents against *Cryptococcus*.[103] The release of granulysin by cytotoxic CD4+ and CD8+ cells and perforin by NK cells probably has a role in the effective killing of *Cryptococcus*.[39,104]

In a mouse model, allergic response and airway reactivity were observed related to pulmonary cryptococcosis.[105] *C. neoformans* specific serum immunoglobulin G (IgG) titers are increased in the serum of some asthma patients in urban areas of Germany, a provocative finding implicating *C. neoformans* in asthma.[106] However, whether pulmonary cryptococcosis is related to either the pathogenesis or exacerbation of asthma is unknown and requires further study.

The fate of *Cryptococcus* within macrophages is determined by the host immune status.[107] *Cryptococcus* cells may be destroyed, resulting in the elimination of infection in most immunocompetent people. Alternatively, *Cryptococcus* may persist in granuloma as sequestrations and establish latent infection in those with alterated immune status or in the presence of specific risk factors.[108] In addition, *Cryptococcus* may colonise the respiratory tract without any symptoms, indicative of incomplete elimination by macrophages.[32] Significantly reduced immunity, especially the CD4 T-helper system, allows *Cryptococcus* to escape from the lung and disseminate.[19,93,109]

Virulence factors of *Cryptococcus*

C. neoformans and *C. gattii* share many virulence factors that allow infection in the human.[30] These virulence factors include polysaccharide capsule, melanin pigments in the cell walls, ability to grow at 37°C, and extracellular enzymes.[110–113] The polysaccharide capsule may disturb T-cell responses in the lung resulting in reduced macrophage functions.[114,115] Oxidative killing of the immune cells against *Cryptococcus* is diminished by melanin with the redox buffer mechanism.[111,116] The survival of *Cryptococcus* at body temperature is critically supported by superoxide dismutase (SOD2), as the null mutant of this gene is both avirulent and loses the ability to grow at 37°C.[112,117] One substantial difference between *C. neoformans* and *C. gattii* is the diminished ability of *C. gattii* to grow inside macrophages.[118]

During pulmonary cryptococcosis caused by *C. neoformans*, cryptococcal titan cells (giant cells) probably play a significant role in host immune evasion.[30] Titan cells are *C. neoformans* cells with an extreme increase in size (25–30 μm in diameter) that leverage the virulence of *C. neoformans* mostly during initial pulmonary infection.[119–121] Mouse models of pulmonary cryptococcosis have shown cryptococcal titan cells can be seen in lung parenchyma after three weeks of infection.[122] Titan cells are not killed by phagocytic activity and exhibit higher resistance to oxidative stress, resulting in a decreased rate of phagocytosis, with reduced pulmonary clearance and possibly dissemination.[119,122,123] Titan cells are a potential novel target cell for therapy of pulmonary cryptococcosis.[121] The published literature is silent on whether titan cells are involved in the pathogenesis of *C. gatti* infection.

Laccase is one of the enzymes contained in the extracellular vesicles of both *C. neoformans* and *C. gattii* and associated with virulence.[113,124] Laccase reduces macrophage activity in the lung and produces melanin pigments, prostaglandin E2 and iron oxidation products.[125–127] These laccase products alter host defences with a decrease in the pulmonary virulence of *Cryptococcus* as observed in a murine study.[128] Cryptococcal laccase leads to a significant decrease in lymphocytes and increase pulmonary eosinophils disturbing the proper adaptive immune response in infected lungs.[120]

Risk factors for pulmonary cryptococcosis

Most patients with pulmonary cryptococcosis (>50%) have no risk factor or immune disorder.[4,6,129–134] Additionally, approximately 60% of pulmonary cryptococcosis cases in the HIV-negative population have no underlying disease.[10,135,136] A report from Thailand of cases collected over 17 years found pulmonary cryptococcosis occurs more frequently in nonimmunocompromised patients than in immunocompromised patients.[137] There were also several reports of co-infection between pulmonary cryptococcosis and tuberculosis in the immunocompetent host, suggestive of some shared, ill-defined risk factor.[138–145]

However, some patients with pulmonary cryptococcosis have a defect in their immune system.[3,49] Hu and colleagues found the genetic polymorphism in Dectin-2 to be more frequent in those with pulmonary cryptococcosis in an HIV-negative population in China.[146] Immunocompromised conditions such as HIV infection, organ transplantation, diabetes mellitus, corticosteroid or immunosuppressive therapy, and malignancy are all conditions linked with pulmonary cryptococcosis.[22,147–149]

HIV infection

A remarkable histopathology autopsy study of 8421 miners who died revealed the incidence of pulmonary cryptococcosis to be 7%, whereas the antemortem incidence was only 2.7%: the estimated HIV-prevalence was 24%.[150] Most of the missed cases were misdiagnosed as pulmonary tuberculosis. *Cryptococcus* was detected from induced sputum in 2.7% pneumonia patients (81% HIV-infected) in Botswana.[151] A study by Park based on global data from United Nations Program on HIV/acquired immunodeficiency disease syndrome (AIDS) 2007 estimated 55% of cryptococcal meningitis patients also have pulmonary cryptococcosis.[152] These studies provide evidence that pulmonary cryptococcosis in HIV patients is underdiagnosed.[151,153,154] There was no significant difference in 90-day and 1-year mortality among HIV patients, organ transplant patients and non-HIV non-organ transplant (NHNT) patients in a cohort study of cryptococcal disease.[148] Pulmonary cryptococcosis was also diagnosed along with other fungal pneumonia such as pulmonary

aspergillosis and *Pneumocystis jirovecii* (*carinii*) lung infections in HIV-positive patients; mixed infection might occur in certain cases.[155]

Organ transplantation

Cryptococcosis is the third most common invasive fungal infection in solid organ transplant (SOT) patients according to data from the US Transplant-Associated Infection Surveillance Network (TRANSNET), among 23 transplantation centres.[156] In renal transplant patients, cryptococcosis is the second most common form of invasive fungal infection with 30-day mortality rate of 19.2%.[156,157] A study from 111 SOT recipients with cryptococcosis, pulmonary cryptococcosis occurred in 54% patients while disseminated cryptococcosis appeared in 61% patients.[158] A large retrospective cohort study of solid organ transplant patients found 46% of 158 cryptococcosis cases to have lungs as the main site of infection; the 90-day mortality rate in SOT patients was 14%.[159] Another study reported that pulmonary cryptococcosis was more likely to occur in SOT recipients from the most recent cohort (2001–2008) than in the previous decades (1960–2000).[160] This study also found nine cryptococcal infections associated with haematological stem cell transplant patients.

Corticosteroid or immunosuppressive therapy

Long-term corticosteroid or immunosuppressive therapy is a risk factor for developing pulmonary cryptococcosis.[10,135,136] In a 35-year retrospective study of pulmonary cryptococcosis in HIV-negative patients, 21% of 151 patients were on corticosteroid therapy.[135] Severe pulmonary sequelae due to pulmonary cryptococcosis were seen after long-term use of corticosteroid.[161,162] Symptomatic pulmonary cryptococcosis was associated with a higher dose of prednisone than those present with asymptomatic pulmonary cryptococcosis in SOT patients.[1]

Malignancies

A recent literature survey found pulmonary cryptococcosis in 26% and 48% in patients with *C. neoformans* infection associated either with underlying disease haematological malignancy or solid organ tumours, respectively.[163] The most common haematological malignancy linked to cryptococcal infection was lymphoma. The case series of cryptococcosis in haematological malignancies revealed the lungs as the infection site in most of the patients.[164] The management of lymphoma with T-cell depleting agents and cancer-related immune alteration are probably responsible. Other reports of HIV-negative patients showed 7.3% and 10.2% of this group had malignancies as risk factors for developing pulmonary cryptococcosis.[10,135]

Diabetes mellitus

Severe diabetes mellitus with organ damage appeared as a major risk factor (31.7%) for pulmonary cryptococcosis among HIV-negative patients.[136,165] Another confirmatory study from Japan confirmed diabetes mellitus as a common underlying disease in the same population, with 32.1% affected among all the cryptococcosis cases.[135] A large study in a diabetes mellitus population with cryptococcosis in China revealed that 12 of 16 (75%) were pulmonary cases.[166] Diabetes was a proven risk factor in a case-control study of cryptococcosis in HIV-negative patients.[167] The mortality rate was higher in cryptococcosis patients with diabetes compare to matched controls.[167] Other immunological disorders that coexist with diabetes mellitus increases the vulnerability of patients to *Cryptococcus*.[168,169]

Cirrhosis

Cirrhosis was reported as a risk factor for cryptococcosis with proportion of pulmonary cryptococcosis in the study population ranged between 18 and 37.5%.[170–172] The 90-days mortality is high at 57.1% with 54.6% of the death is associated with cryptococcosis.[171] Although there are case reports of pulmonary cryptococcosis with cirrhosis the correlation between these two diseases is still unknown.[161,173,174]

Clinical manifestation of pulmonary cryptococcosis

The central nervous system (CNS) and the lung are the two organs that comprise the main sites of *Cryptococcus* infection.[84] However, skin, soft tissue, joint, bone, kidney, muscle, liver, kidney, and other organs may also be infected by this fungus.[175–178] Among non-HIV patients, pulmonary cryptococcosis is the most common non-CNS location.[179]

Pulmonary cryptococcosis usually presents with nonspecific symptoms such as cough, dyspnea, chest pain and fever both in adults and children.[6,13,22,49,135,149,180–185] However, it may be totally asymptomatic or may present with respiratory failure[22,49,182,186,187] (Table 2). The nonspecific symptoms of this disease are likely to cause delays in diagnosis and proper treatment resulting in further dissemination of *Cryptococcus* infection.[166,182,188] Physical examination may reveal reduced breath sounds, crackles, and/or dullness to percussion indicated pleural effusion.[189] *Cryptococcus* infection leads to differing clinical manifestations in immunocompromised and immunocompetent people.[182] Approximately half of immunocompetent patients with pulmonary cryptococcosis are asymptomatic, the infection incidentally detected during a routine chest X-ray (CXR) or follow-up of other diseases.[133,135,149] In contrast, it is very rare that immunocompromised patients experience asymptomatic pulmonary cryptococcosis,[182] apart from the rare asymptomatic solitary pulmonary nodules in HIV patients.[190] Acute respiratory failure was the presentation in 33% of pulmonary cryptococcosis cases reported without HIV, most of whom had a solid organ transplant as their underlying condition.[168] In all probability many other factors affect the clinical appearance of pulmonary cryptococcosis such as geography, virulence of different

Table 2. Clinical manifestations of pulmonary cryptococcosis.

	Cryptococcus neoformans		*Cryptococcus gattii*	
Symptoms	Immunocompromised	Immunocompetent	All subjects	References
Asymptomatic	–	24–54%	34%	6,135,149,182,183
Fever	66%	15–63%	42%	6,135,180,183,184,275
Cough	58–66%	17–62%	56%	6,180,183,184,275
Dyspnea	33–50%	22–48%	51%	6,135,183,184,275
Chest pain	25%	7–44%	28%	6,180,181,183,184,275

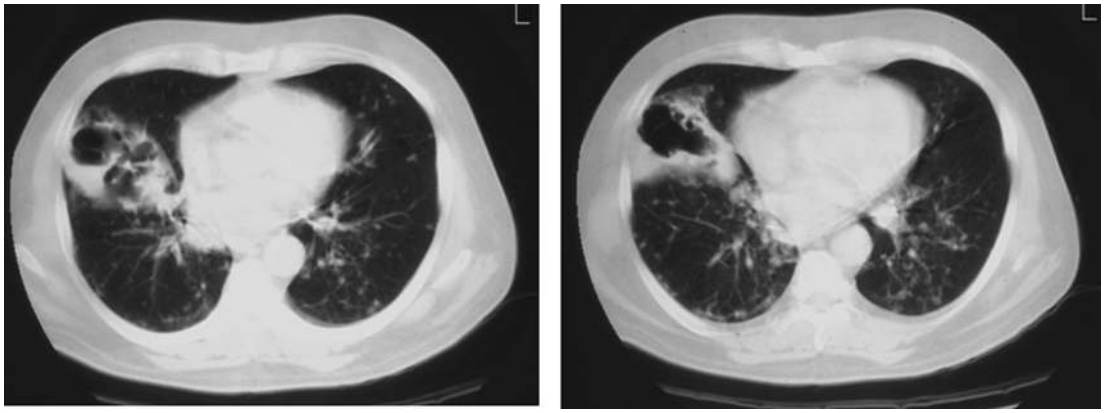

Figure 1. Pulmonary cryptococcosis appearances in chest-computed tomography scans show infective inflammatory changes with cavitations and discrete nodules.

Cryptococcus strains, and possibly immunogenetic variations among patients.[54,191]

Radiological appearances of pulmonary cryptococcosis

The radiological appearance of pulmonary cryptococcosis may mimic other clinical conditions such as other pulmonary infections caused by bacteria, mycobacteria, parasites, or viruses, malignancy, inflammatory reaction, abscesses, malignancy and infarction.[192–195] Nodules, especially located in the peripheral area of the lung, were the most commonly diagnosed finding on CXR and computed tomography (CT) scan of noncompromised patients with proven pulmonary cryptococcosis[4,5,10,129,133,196,197] (Fig. 1). Nodules may be single or multiple.[198] Pulmonary cryptococcosis has often been initially misdiagnosed as lung cancer as some nodules also were positive on F-fluorodeoxyglucose positron emission tomography (FDG-PET) (Fig. 2).[4,5,199] Rarer appearances of pulmonary cryptococcosis include calcification, "tree in bud" appearances, lymphadenopathies, and pleural effusions.[5]

In contrast, immunocompromised patients showed a broad spectrum of abnormal CXR and CT scan appearances including both single or multiple nodules, segmental consolidation, cavitation, bilateral bronchopneumonia, mass-like appearances, a diffuse miliary pattern, or mixed patterns.[182,197,200,201] The

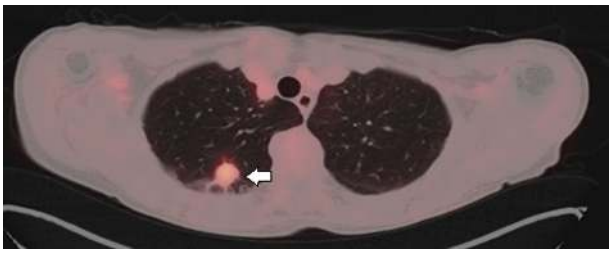

Figure 2. PET/CT scans from proven pulmonary cryptococcosis showing a spiculated mass (arrows) with high F-fluorodeoxyglucose (FDG) uptake resembles lung cancer mass. This Figure is reproduced in color in the online version of *Medical Mycology*.

CT appearances of proximal air bronchogram, cavitation, and halo sign were more typically seen in immunocompromised patients than noncompromised patients.[4,10,129] Immunocompetent patients more often appeared with solitary and well-defined nodules.[10,202,203]

Laboratory diagnosis of pulmonary cryptococcosis

Given the complexities surrounding the diagnosis and identification of pulmonary cryptococcosis, the diagnosis of pulmonary cryptococcosis is usually based on a combination of clinical and radiological suspicion and laboratory confirmation.[15,166,189,192,204–208] The methods used to confirm the infection are culture, direct microscopic, histopathology, serology,

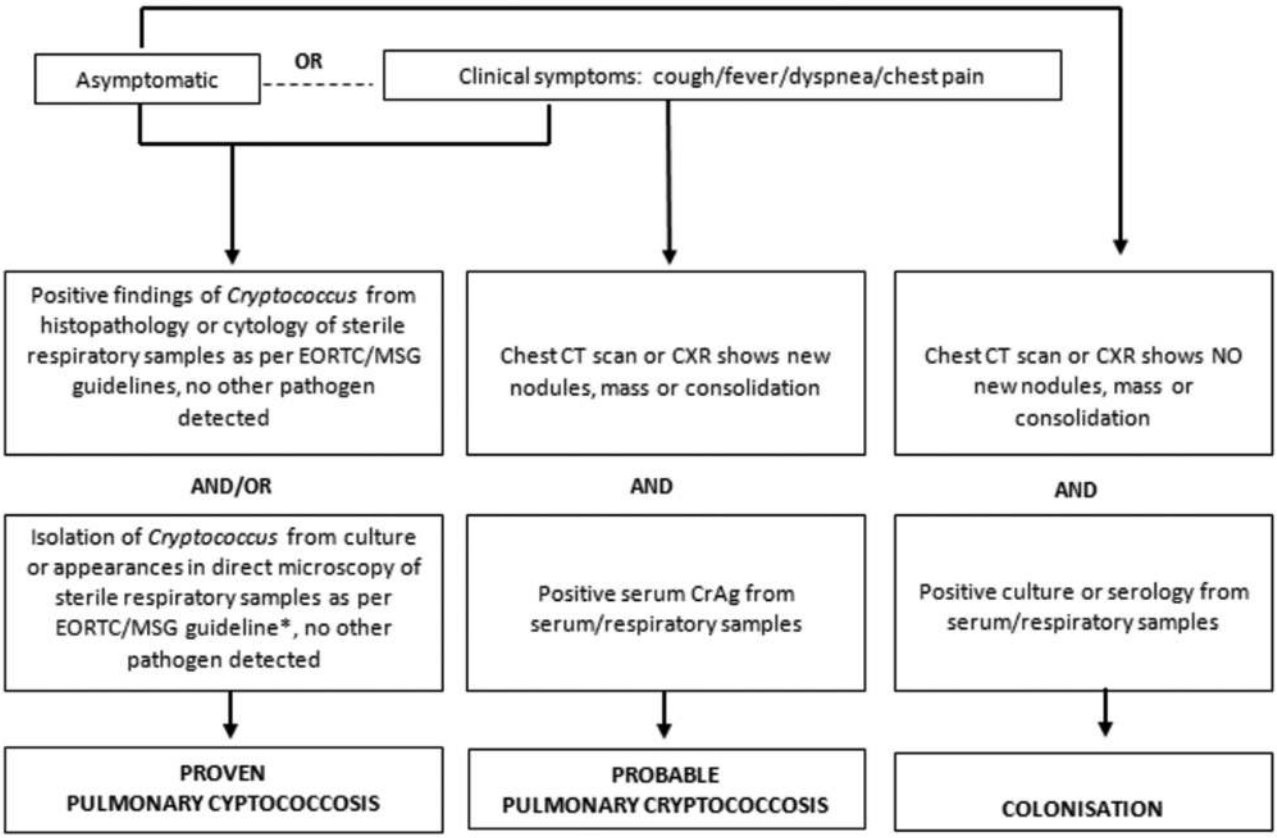

Figure 3. Algorithm for the diagnosis of pulmonary cryptococcosis. (Modified from Pauw et al.190) EORTC/MSG, European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group; CrAg, *Cryptococcus* antigen; CT, computed tomography; CXR, chest x-ray.

and molecular detection.[189,204,205,208,209] We propose diagnostic criteria for pulmonary cryptococcosis in Figure 3. Identifying a positive culture of *Cryptococcus* from bronchoalveolar lavage (BAL) or pleural fluid, together with appropriate clinical symptoms and/or radiology findings are the key diagnostic approaches.[49,210] These findings may be discovered in parallel with, or without, positive antigen tests or direct microscopy from serum, BAL or pleural fluid.[210,211] Antigen tests from serum or blood or culture are rarely positive unless there is disseminated cryptococcal infection.[212] The use of lysis centrifugation of the buffy coat from blood may increase detection.[213] Once a diagnosis of pulmonary cryptococcosis is made, a lumbar puncture and cerebrospinal fluid (CSF) examination (including antigen) are recommended in all patients.[214] The differential diagnosis of pulmonary cryptococcosis is summarized in Table 3.

Microscopy and culture

Sputum, BAL, pleural fluid, or tissue from a lung biopsy is collected for microscopy to visualize yeasts and fungal culture may grow *Cryptococcus* spp.[22,213] A lung biopsy from nodules of uncertain aetiology requires a fungal culture to be done, in addition to histopathology examination. The laboratory procedure for handling sputum from suspected cases is not standardised, due to different culture conditions and media used by local laboratories.[22] The pellet from pleural fluid or BAL can be mixed with India ink and observed under a microscope. The distinctive structure for *Cryptococcus* spp. is narrow budding encapsulated yeasts.

Samples for culture should be placed on Sabouraud dextrose agar at 30°C for 7 days, in aerobic conditions, and observed daily.[22,189] Cultures from patients receiving systemic antifungal therapy might need longer to grow *Cryptococcus*.[189] *Cryptococcus* appears as mucoid creamy colonies. *C. neoformans* are identified generally as smooth colonies while *C. gattii* mostly appears as mucoid colonies.[215,216]

Canavanine-glycine-bromothymol blue (CGB) agar can be used to differentiate between *C. neoformans* and *C. gattii*.[49,213] Colonies of *C. neoformans* will not cause changes in CGB agar. On the other hand, *C. gattii* produces a blue color in CGB agar. Unlike other types of specimens, there are many variations in the culture media and culture conditions for sputum. One study reported that birdseed extract (BSE) agar may increase *Cryptococcus* detection in sputum and urine samples in HIV patients,[217] partly because white colonies on Sabouraud dextrose agar may be indistinguishable from *Candida* spp. The addition of benomyl to BSE agar has been shown to likely increase detection

Table 3. Differential diagnosis of pulmonary cryptococcosis.*

Condition	Similarity with pulmonary cryptococcosis	Differentiating test
Bacterial, mycobacterial, viral, or other pulmonary fungal infections	• The same signs and symptoms	• BAL/ Sputum microscopy and culture: positive for specific organism
• Bacteria: *Fusobacterium, Pseudomonas, Streptococcus*	• The same radiological appearances	• Serum/BAL CrAG test
• Atypical pathogen and Mycobacteria: *Mycoplasma pneumoniae, Chlamydia pneumoniae, and Legionella pneumophila* Mycobacterium tuberculosis, M. kansasii		• Detection of mycobacteria using GenXpert TB or Ziehl-Neelsen stain to show acid-fast bacilli: to differentiate mycobacteria
• Virus: *cytomegalovirus*		• Immunofluorescence, Gomori methenamine, or Wright-Giemsa staining of sputum/bronchoalveolar lavage
• Other pulmonary fungal infections: *Aspergillus, Pneumocystis, Histoplasma, Coccidioides*		• HIV and CD4 count
Primary lung cancer or metastatic neoplasm	• The same radiological appearances	• CT or MRI of the lung or other organ shows presence of primary or metastatic tumour(s)
	• Could be asymptomatic at all	• Tissue biopsy: malignant cells

*Adapted from Limper et al.[15]; Li et al.[166]; Riha, Pataka.[189]; Guimares et al.[192]; Guarner, Brandt.[206]; Thibodeau, Viera.[207]; and Shibuya et al.[208]

of *Cryptococcus* from environmental isolates when the samples are highly contaminated with other moulds.[218] Benomyl is a fungicide that inhibits ascomycetes but has no effect on basidiomycetes including *C. neoformans* and *C. gattii*. Positive results for *C. neoformans* and *C. gattii* cultures from any types of specimens should be followed by clinical investigation.[22]

Histopathology

A lung biopsy is the best diagnostic option when sputum or bronchoscopy specimens are unavailable or negative.[22] There are several methods of lung biopsy including percutaneous lung biopsy, trans-bronchial lung biopsy, video-assisted thoracoscopic (VAT) biopsy, and open lung biopsy. The most appropriate method to use for the biopsy depends on the skills available and the location of the lung lesion.

Histological staining with hematoxylin and eosin (H&E), Grocott or Gomori methenamine silver (GMS), and periodic acid-Schiff (PAS) are used to detect *Cryptococcus* that appears as narrow-based budding yeasts (4-10 μm), usually surrounded by thick capsules in the lung tissue.[49,206] Tissue sections can be processed with alcian blue or mucicarmine to display the capsule[213] (Fig. 4). Mucicarmine can differentiate *Cryptococcus* from other yeast-like structures such as those of *Coccidioides*, *Histoplasma*, or *Candida*, but only stain *Blastomyces* weakly.[219] Occasionally, *Cryptococcus* infection with a capsule-deficient strain is present and not detected with mucicarmine.[212,220] In this instance, Fontana-Masson stain should be used to detect *Cryptococcus* because it stains melanin and other silver-reducing granules in the *Cryptococcus* cell walls.[109,221] Several studies have reported that the majority of the *Cryptococcus* samples revealed a lack of capsule and instead unusual structures (for *Cryptococcus*) such as pseudohyphae, germ tubes and chains of budding yeasts.[212,222] In addition, titan cells were identified in 10% of pulmonary cryptococcosis cases.

A wide variety of inflammatory reactions are observed in pulmonary cryptococcal infection from well-formed granulomas to minimal inflammation.[204] A complete granuloma complex is built from aggregates of macrophages, lymphocytes, plasma cells, and multinucleated giant cells.[223] The presence and architecture of granulomas or other lesions are likely related to *Cryptococcus* species (and capsule characteristics) and host immune status.[49,206] Postmortem studies of HIV patients with pulmonary cryptococcosis have revealed abundant organism in distended alveoli, submucosa, and peribronchial tissue without any sign of an inflammatory response,[224] whereas typical granulomas and other inflammatory cells comprised the majority of the histopathology findings from immunocompetent patients.[49,225]

Positive histopathology does not always correlate with the culture result. A negative culture might be caused by nonviable organisms in the sample. Immune responses in the lungs, especially granuloma formation, may render *Cryptococcus* nonviable.[2,226,227]

Antigen detection tests

Capsular polysaccharides of *Cryptococcus* can be detected and quantified from body fluids such as serum, CSF, BAL, and

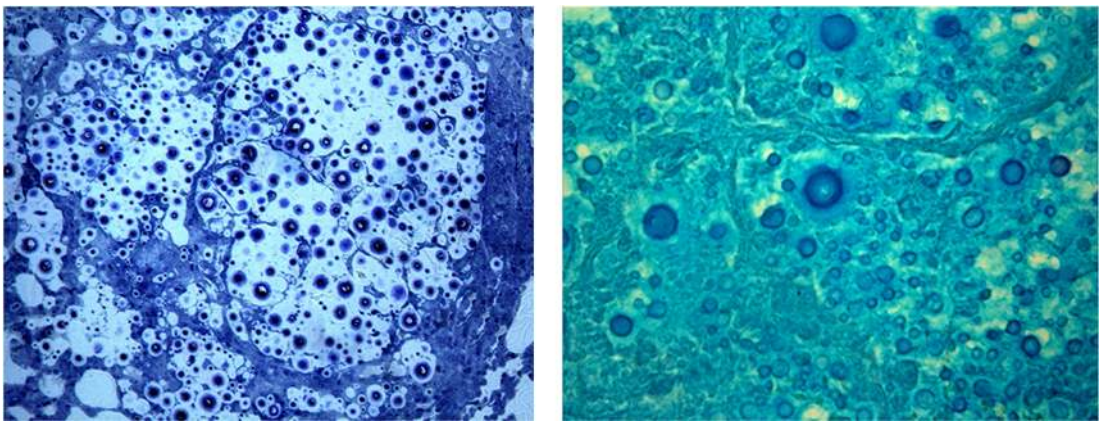

Figure 4. *Cryptococcus neoformans* in the murine lung with toluidine blue stain (left); *Cryptococcus neoformans* in the human lung with alcian blue stain (right). This Figure is reproduced in color in the online version of *Medical Mycology*.

urine using specific anti-C. *neoformans* antisera.[191,219] Three formats of cryptococcal antigen (CrAg) detection tests are currently available: the latex agglutination test (LAT), the enzyme-linked immunoassay (EIA), and the lateral flow immunoassay (LFA).[191,228,229] These methods are rapid, sensitive and specific but have not been standardised for respiratory specimens such as BAL, pleural fluid, or sputum. The sensitivity of serum CrAg for cryptococcal meningitis and disseminated disease is 93–100%, and the specificity is 93–98%.[189] The sensitivity and specificity of CSF CrAg are even higher. False negative serum CrAg is commonly associated with pulmonary cryptococcal infection, probably because of the low fungal burden outside the lung or the capsule-deficient strain of *Cryptococcus*.[134] However, it may be positive in disseminated cryptococcosis with lung involvement.[135,189,210] False-positive results are seen in the presence of rheumatoid factors or infections by *Klebsiella pneumonia, Trichosporon beigelii, Stomatococcus mucilaginosus,* or *Capnocytophaga canimorsus*.[189]

The median titer of serum CrAg in pulmonary cryptococcosis in non-HIV patients is 1:16 in patients without any underlying diseases, and 1:32 in those with some form of immunocompromised state.[135] In a 12-month follow-up, serum CrAg titers decrease slowly on therapy from >1:32 to negative in most (76%) immunocompetent patients suffering from pulmonary cryptococcosis.[2] However, the decrease is also seen in untreated survivors although the CrAg rarely becomes negative. At the same time, CrAg may persist for months after successful therapy. Therefore, serum CrAg cannot be used as a prognostic tool or for assessing response to treatment for pulmonary cryptococcosis.

Several studies report the use of BAL and percutaneous pulmonary aspirates for CrAg detection.[230–233] Detection of CrAg using BAL of HIV-positive patients shows 100% sensitivity and 98% specificity with CrAg titer of ≥1:8 as a detection limit.[230,231] This is in line with findings from immunocompetent patient lung aspirates showing 100% sensitivity and 97% specificity.[232] False negatives may be caused by dilution of the sample in the process of bronchial washing. A recent study with 23 HIV-negative pulmonary cryptococcosis patients revealed 82.6% sensitivity of CrAg detection in BAL, while the serum CrAg sensitivity was only 73.9%.[234] Latex agglutination methods have been used for the detection of CrAg in respiratory samples in all published studies. The performance of the LFA in the diagnosis of pulmonary cryptococcosis has not been evaluated. Although BAL and lung aspirate have potential as a diagnostic tool, bronchoscopy is an invasive method. It is unlikely bronchoscopy will be performed unless the patient presents with nodules and there is a suspicion of malignancy or the clinical presentation is severe. Sputum may prove to be an alternative sample type for early diagnosis of pulmonary cryptococcosis as *Cryptococcus* cells can be detected both in sputum and lung aspirates by cytology.[235]

Molecular detection

Molecular detection of *Cryptococcus* is required in specific situations where other diagnostic tools have failed to confirm a diagnosis of cryptococcosis.[219] One of the circumstances is when there is positive result from histological examination but the culture is negative. These molecular methods include pan-fungal PCR, DNA sequencing for identification, multiplex PCR, isothermal amplification method, and probe-based microarrays.[236–238]

The conventional method for *Cryptococcus* species differentiation is a CGB test. However, studies revealed that CGB test is inferior than PCR in distinguishing species of *Cryptococcus* in 4.58% of samples.[239,240] Moreover, it provides rapid identification and may identify mating-type profiles via amplification of the STE (sterile) gene sequences.[241] There are several studies that have used PCR for the direct detection of *Cryptococcus* spp. in respiratory samples (Table 4).[236–239,242–246] Respiratory samples from proven pulmonary cryptococcocosis processed with multiplex RT PCR showed 90.7% sensitivity and 100% specificity to detect C. *neoformans*.[236] PCR may also distinguish different

Table 4. Review of PCR methods for *Cryptococcus* spp.

No.	PCR method	Samples	DNA extraction	Primer/target gene	Accuracy and LoD	Reference
1	Singleplex PCR (*Cryptococcus neoformans var. grubii, C. neoformans var. neoformans, C. gattii* and *hybrids)*	References strain	–	STR1F and STR1R	Sensitivity: 99.2%	237
2	PCR and restriction digest (*C. neoformans* and *C. gattii)*	Clinical isolates collection	Thermal shock method	ITS4-ITS5 continue with restriction digest with NS7-ITS2 and NS7-ITS4	–	242
3	Nested PCR (*C. neoformans*)	Sputum, BAL, biopsy, bronchial aspirates from PC patients	glass bead technique and heat treatment	URA5 gene	10 pg; 1×10^3 cfu	243
4	Multiplex PCR (serotype and mating type of *C. neoformans)*	Pigeon droppings	glass bead technique	STE20 gene	–	244
5	Nested and real time PCR	Brain from infected mice	QIAamp tissue kit	rRNA (18S rDNA)	1 to 10 cfu	245
6	Multiplex PCR (*C. gattii*, *C. neoformans var neoformans* and *var. grubii)*	Clinical isolates recovered by culture	CTAB technique	CNa-70S and CNa-70A for *C. neoformans*, CNb-49S and CNb-49A for *C. gattii*	1.25 ng, 100% agreement with serotyping	239
7	Multiplex RT PCR (*C. neoformans*)	Clinical samples (BAL, sputum, blood)	–	OLI CRYPTO 1 2 OLI CRYPTO 2	2 fg, sensitivity: 90.7%, specificity: 100%	236
8	Nested PCR (*C. neoformans*)	Cerebrospinal fluid	chloroform technique	First run: ITS-1and CN-4 Second run: CN-5 and CN-6	10 cells	246
9	Comparison primer sets (*C. gattii* and *C. neoformans*)	Cerebrospinal fluid	QIAamp DNA mini kit	CN4–CN5 (coding rDNA) & the multiplex CNa70S–CNa70A/CNb49S–CNb-49A	Sensitivity: 94.8% Specificity: 98.5%	238

BAL, bronchoalveolar lavage; cfu, colony-forming unit; CTAB, cetyl trimethylammonium bromide DNA, deoxyribonucleic acid; LoD, limit of detection; PC, pulmonary cryptococcosis; PCR, polymerase chain reaction; RT, real-time.

species of *Cryptococcus* spp. using STR1F and STR1R as the target genes.[237]

Management of pulmonary cryptococcosis

Successful management of pulmonary cryptococcosis depends on the host-immune status and the predilection of the disease, whether present as a localised disease in the lung, spread to CNS, or generally disseminated (Table 5).[211,214,247] Both immunocompromised and immunocompetent patients with cryptococcal pneumonia should have blood culture, CSF examination via lumbar puncture, and serum cryptococcal antigen testing.[22] Whenever CNS involvement is identified, this requires induction amphotericin B or fluconazole combined with flucytosine, and the adjustment of the dosage and duration of antifungal therapy, compared to pulmonary involvement alone.[214,247,248] Although flucytosine is now clearly established as a critical therapy for cryptococcal meningitis, its short half-life and time over minimum inhibitory concentration (MIC) pharmacodynamic requires 6-hourly dosing in those with normal renal function. This is problematic and a slow release formulation would be welcome.[248]

Fluconazole is the drug of choice for cryptococcosis without CNS involvement.[249] One study reported that eight of 25 (32%) nonimmunocompromised patients experienced spontaneous recovery from isolated pulmonary cryptococcosis without antifungal treatment.[250] A recent literature review of immunocompetent patients recommends fluconazole 400 mg/day

Table 5. Summary of antifungal therapy for pulmonary cryptococcosis.*

HIV and immunocompromised patients		
Asymptomatic, Mild disease, and/or positive culture	Fluconazole 400–800 mg/d or itraconazole 400 mg/d for 6–12 months, followed by maintenance	Maintenance discontinued after HAART therapy if disease free and CD4 count > 200 cells/μl for
Maintenance	Fluconazole (200 mg/d)	
Immunocompetent		
Colonized	No specific antifungal therapy	
Symptomatic, mild-moderate disease	Fluconazole (400–800 mg/d initially), for 6–12 months or itraconazole (400 mg/d for 6 months)	Suggested extended duration if the response is not complete

HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus.
*Adapted from Perfect et al.[214] and Limper et al.[247]

orally for six to twelve months in either symptomatic or asymptomatic conditions.[2] This is because antifungal therapy prevents or diminishes the risk of disease progression. Oral itraconazole or voriconazole 200 mg twice daily may be substituted if fluconazole is not available or contraindicated.[247] It may be necessary to add corticosteroid in cases of acute respiratory distress syndrome.[214]

A recent study described a 10.6% mean fluconazole resistance from 4995 *Cryptococcus* isolates from HIV-positive patients.[251] The rate of fluconazole resistance is increasing over time which is an alarming rate for cryptococcosis management.[181,252] There is an urgent need for novel antifungal to counter the resistance strains of *Cryptococcus*.[253] APX001A, VT-1129, and T-2307 are being developed as the new therapeutic options for cryptococcosis. APX001A already completed phase II clinical trial, while VT-1129 and T-2307 developed passed phase I clinical trial.[254–256] T-2307 may have value in pulmonary cryptococcosis as in a murine model as it prevents alveolar collapse, while this important activity is not observed with fluconazole and amphotericin B.[255] Furthermore, established therapeutic drugs such as sertraline, amiodarone, thioridazine and tamoxifene are repurposed as anticryptococcal agents. Sertraline, a selective serotonin reuptake inhibitor, is under phase III clinical trial for adjuvant therapy of cryptococcosis.[257] Amiodarone and thioridazine show active killing of intraphagocyte *C. neoformans* in the lungs known as an inaccessible location for fluconazole and amphotericin B.[258]

Prognosis of pulmonary cryptococcosis

Pulmonary cryptococcosis lead to no deaths, relapses, or dissemination among non-HIV patients in China, with a follow-up of 2 to 11 years.[149] However, acute severe respiratory distress is a possible outcome of pulmonary cryptococcosis both in HIV or non-HIV patients.[168,186,259,260] A 10-year retrospective study concerning non-HIV pulmonary cryptococcosis reported that 33% of patients developed respiratory failure, with a 55% mortality rate.[168] All cases of respiratory failures occurred within twenty-four hours of the patient arriving at the hospital. The mortality rate of pulmonary cryptococcosis in HIV patients in one study was 74% over the first year following diagnosis.[261] All of the patients who did not survive had disseminated infection. A recent study of pulmonary cryptococcosis in both immunocompromised and non-compromise patients showed 70.3% ($n = 71$) were successfully treated.[129]

Prevention of pulmonary cryptococcosis

Primary antifungal prophylaxis for cryptococcosis in HIV patients is only recommended for regions with a high rate of disease, high levels of antiretroviral drugs resistance, and restricted availability of highly active antiretroviral therapy (HAART).[214] Randomised controlled trial studies found fluconazole as a first line prophylaxis, or itraconazole as a second line prophylaxis, for HIV patients with low CD4 counts effective.[262–264]

Other prevention methods might include *Cryptococcus* vaccines, although none are yet licensed.[265–268] This approach has potential value because of the high risk of the antifungal toxicity, the rise of antifungal resistances in *Cryptococcus*, and immune defects that hamper *Cryptococcus* eradication.[181,269,270] A recent preclinical study in mice utilising vaccines from the antigen extracts of *Cryptococcus* coated with glucan particles indicated potential advantages with a 60% 50-day survival rate.[268] Other studies using different models of *Cryptococcus* vaccines also support this approach as part of strategy to prevent pulmonary cryptococcosis.[267,271–273]

Pulmonary cryptococcosis is probably one of the most common pulmonary fungal infections both in immunocompromised and immunocompetent patients. There has been increasing interest in the field of genetic diversity, environmental niches and the pathogenesis of this disease, especially since the outbreak of *C. gattii* in several areas. However, most of the studies are small; hence, caution must be applied as the findings may not be transferable to different geographical areas or patient risk groups. The development of diagnostic tools or point of care test that can detect CrAg in respiratory specimens is the substantial part of research gap in pulmonary cryptococcosis.[234] The interpretation

of CrAg in respiratory specimens required support from clinical appearance as colonization is frequently occurs in respiratory tracts.[274] Further research is needed to account for the varying risk factors, including genetic risk factors, embracing a greater sample size, especially in relation to immunocompetent populations. There is also limited clinical research involving patients with isolated pulmonary cryptococcosis. Thus, further research is required to determine the best strategy for rapid diagnosis of pulmonary cryptococcosis to preempt dissemination and acute respiratory failure.

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Declaration of interest

D.W.D. and family hold Founder shares in F2G Ltd., a University of Manchester spin-out antifungal discovery company. He acts or has recently acted as a consultant to Astellas, Sigma Tau, Basilea, Scynexis, Cidara, Biosergen, Quintiles, Pulmatrix, Pulmocide, and Zambon. In the last 3 years, he has been paid for talks on behalf of Astellas, Dynamiker, Gilead, Merck, and Pfizer. He is a longstanding member of the Infectious Disease Society of America Aspergillosis Guidelines group, the European Society for Clinical Microbiology and Infectious Diseases Aspergillosis Guidelines group, and the British Society for Medical Mycology Standards of Care committee. R.R.-R. reports personal fees from Gilead Sciences and Astellas. F.S. declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References

1. Singh N, Alexander BD, Lortholary O et al. Pulmonary cryptococcosis in solid organ transplant recipients: clinical relevance of serum cryptococcal antigen. *Clin Infect Dis*. 2008; 46: e12–e18.
2. Fisher JF, Valencia-rey PA, Davis WB. Pulmonary cryptococcosis in the immunocompetent patient — many questions, some answers. *Open Forum Infect Dis*. 2016; 3: 1–6.
3. Rigby AL, Glanville AR. Miliary pulmonary cryptococcosis in an HIV-positive patient. *Am J Respir Crit Care Med*. 2012; 186: 200–201.
4. Zhang Y, Li N, Zhang Y et al. Clinical analysis of 76 patients pathologically diagnosed with pulmonary cryptococcosis. *Eur Respir J*. 2012; 40: 1191–1200.
5. Qu Y, Liu G, Ghimire P et al. Primary pulmonary cryptococcosis: evaluation of CT characteristics in 26 immunocompetent Chinese patients. *Acta Radiol*. 2012; 53: 668–674.
6. Ye F, Xie J, Zeng Q, Chen G, Zhong S, Zhong N. Retrospective analysis of 76 immunocompetent patients with primary pulmonary cryptococcosis. *Lung*. 2012; 190: 339–346.
7. Willenburg KS, Hadley S. Pulmonary cryptococcosis: A rare but emerging disease. *Curr Fungal Infect Rep*. 2009; 3: 40–44.
8. Yoo SD, Worodria W, Med M et al. The prevalence and clinical course of HIV-associated pulmonary cryptococcosis in Uganda. *J Acquir Immune Defic Syndr*. 2011; 54: 269–274.
9. Harris JR, Lindsley MD, Henchaichon S et al. High prevalence of cryptococcal infection among HIV-infected patients hospitalized with pneumonia in Thailand. *Clin Infect Dis*. 2012; 54: 43–50.
10. Liu K, Ding H, Xu B et al. Clinical analysis of non-AIDS patients pathologically diagnosed with pulmonary cryptococcosis. *J Thorac Dis*. 2016; 8: 2813–2821.
11. Suwatanapongched T, Sangsatra W, Boonsarngsuk V, Incharoen P. Clinical and radiologic manifestations of pulmonary cryptococcosis in immunocompetent patients and their outcomes after treatment. *Diagnostic Interv Radiol*. 2013; 19: 438–446.
12. Sun L, Chen H, Shao C, Song Y, Bai C. Pulmonary cryptococcosis with trachea wall invasion in an immunocompetent patient: a case report and literature review. *Respiration*. 2014; 87: 324–328.
13. Gao L-W, Jiao A-X, Wu X-R et al. Clinical characteristics of disseminated cryptococcosis in previously healthy children in China. *BMC Infect Dis*. 2017; 17: 359.
14. Cao W, Jian C, Zhang H, Xu S. Comparison of clinical features and prognostic factors of cryptococcal meningitis caused by *Cryptococcus neoformans* in patients with and without pulmonary nodules. *Mycopathologia*. 2018: 1–8.
15. Limper AH. The changing spectrum of fungal infections in pulmonary and critical care practice: clinical approach to diagnosis. *Proc Am Thorac Soc*. 2010; 7: 163–168.
16. Chen M, Xu Y, Hong N et al. Epidemiology of fungal infections in China. *Front Med*. 2018; 12: 58–75.
17. Wu SX, Guo NR, Li XF et al. Human pathogenic fungi in China-emerging trends from ongoing national survey for 1986, 1996, and 2006. *Mycopathologia*. 2011; 171: 387–393.
18. Chau TTH, Mai NH, Phu NH et al. A prospective descriptive study of cryptococcal meningitis in HIV uninfected patients in Vietnam - high prevalence of *Cryptococcus neoformans var grubii* in the absence of underlying disease. *BMC Infect Dis*. 2010: 2–9.
19. Voelz K, May RC. Cryptococcal interactions with the host immune system. *Eukaryot Cell*. 2010; 9: 835–846.
20. May RC, Stone NRH, Wiesner DL, Bicanic T, Nielsen K. *Cryptococcus* : from environmental saprophyte to global pathogen. *Nat Rev Microbiol*. 2015; 14: 106–117.
21. Mitchell TG, Castaneda E, Nielsen K, Wanke B, Lazera MS. Enviromental niches for *Cryptococcus neoformans* and *Cryptococcus gattii*. In: Heitman J, Kozel T, Kwon-Chung KJ, Perfect JR, Casadevall A, eds. *Cryptococcus: From Human Pathogen to Model Yeast*. Washington, DC: ASM Press, 2011: 237–260.
22. Chang CC, Sorrell TC, Chen SCA. Pulmonary cryptococcosis. *Sem Respir Crit Care Med*. 2015; 36: 681–691.
23. Chowdhary A, Rhandhawa HS, Prakash A, Meis JF. Environmental prevalence of *Cryptococcus neoformans* and *Cryptococcus gattii* in India: an update. *Crit Rev Microbiol*. 2012; 38: 1–16.
24. Springer DJ, Billmyre RB, Filler EE et al. *Cryptococcus gattii* VGIII isolates causing infections in HIV/AIDS patients in southern California: identification of the local environmental source as arboreal. *PLoS Pathog*. 2014; 10: e1004285.
25. Sampaio A, Sampaio JP, Leão C. Dynamics of yeast populations recovered from decaying leaves in a nonpolluted stream: A 2-year study on the effects of leaf litter type and decomposition time. *FEMS Yeast Res*. 2007; 7: 595–603.
26. Baroni FDA, Paula CR, Da Silva ÉG et al. *Cryptococcus neoformans* strains isolated from church towers in Rio de Janeiro City, RJ, Brazil. *Rev Inst Med Trop Sao Paulo*. 2006; 48: 71–75.
27. Ergin C, Ilkit M, Kaftanog O. Detection of *Cryptococcus neoformans var.grubii* in honeybee (*Apis mellifera*) colonies. *Mycoses*. 2004; 47: 431–434.
28. Russo G, Libkind D, Sampaio JP, Van Broock MR. Yeast diversity in the acidic Rio Agrio-Lake Caviahue volcanic environment (Patagonia, Argentina). *FEMS Microbiol Ecol*. 2008; 65: 415–424.
29. De García V, Brizzio S, Libkind D, Buzzini P, Van Broock M. Biodiversity of cold-adapted yeasts from glacial meltwater rivers in Patagonia, Argentina. *FEMS Microbiol Ecol*. 2007; 59: 331–341.
30. Kronstad JW, Attarian R, Cadieux B et al. Expanding fungal pathogenesis: *Cryptococcus* breaks out of the opportunistic box. *Nat Rev Microbiol*. 2011; 9: 193–203.
31. Malik R, Krockenberger MB, Brien CRO, Carter DEEA, Meyer W, Canfield PJ. Veterinary insights into cryptococcosis caused by *Cryptococcus neoformans* and *Cryptococcus gattii*. In: Heitman J, Kozel T, Kwon-Chung KJ, Perfect JR, Casadevall A, eds. *Cryptococcus: From Human Pathogen to Model Yeast*. Washington, DC: ASM Press, 2011: 489–502.
32. Lin X, Heitman J. The biology of the *Cryptococcus neoformans* species complex. *AnnuRevMicrobiol*. 2006; 60: 69–105.

33. Sethi K, Randhawa H. Survival of *Cryptococcus neoformans* in the gastrointestinal tract of pigeons following ingestion of the organism. *J Infect Dis*. 1968; 118: 135–138.
34. Fonseca Á, Boekhout T, Fell JW. *Cryptococcus Vuillemin (1901)*. Vol 3. New York: Elsevier B.V., 2011.
35. Kwon Chung K, Boekhout T, Wickes B, Fell J. Systematics of the genus *Cryptococcus* and its type species *C. neoformans*. In: Heitman J, Kozel T, Kwon-Chung KJ, Perfect JR, Casadevall A, eds. *Cryptococcus: From Human Pathogen to Model Yeast*. Washington, DC: ASM Press, 2011: 3–15.
36. Desnos-Ollivier M, Patel S, Raoux-Barbot D, Heitman J, Dromer F. Cryptococcosis serotypes impact outcome and provide evidence of *Cryptococcus neoformans* speciation. *MBio*. 2015; 6: e00311–15.
37. Deepa S, Felipe H S-T, Tamara L D. *Cryptococcus neoformans*: Historical curiosity to modern pathogen. *NIH Public Access Yeast*. 2014; 31: 47–60.
38. Khawcharoenporn T, Apisarnthanarak A, Mundy LM. Non-neoformans cryptococcal infections: a systematic review. *Infection*. 2007; 35: 51–58.
39. Ma H, May RC. Virulence in *Cryptococcus* species. In: *Advances in Applied Microbiology*. Vol 67. New York: Elsevier, 2009: 131–190.
40. de Araujo GS, Fonseca FL, Pontes B et al. Capsules from pathogenic and non-pathogenic *Cryptococcus* spp. manifest significant differences in structure and ability to protect against phagocytic cells. *PLoS One*. 2012; 7.
41. Takemura H, Ohno H, Miura I, Takagi T. The first reported case of central venous catheter-related fungemia caused by *Cryptococcus liquefaciens*. *J Infect Chemother*. 2015; 21: 392–394.
42. Conde-Pereira C, Rodas-Rodriguez L, Diaz-Paz M et al. Fatal case of polymicrobial meningitis caused by *Cryptococcus liquefaciens* and *Mycobacterium tuberculosis* complex in a human immunodeficiency virus-infected patient. *J Clin Microbiol*. 2015; 53: 2753–2755.
43. Nielsen K, De Obaldia AL, Heitman J. *Cryptococcus neoformans* mates on pigeon guano: Implications for the realized ecological niche and globalization. *Eukaryot Cell*. 2007; 6: 949–959.
44. Walter JE, Yee RB. Factors that determine the growth of *Cryptococcus neoformans* in avian excreta. *Am J Epidemiol*. 1968; 88: 445–450.
45. Staib F, Grave B, Altmann L, Mishra SK, Abel T, Blisse A. Epidemiology of *Cryptococcus neoformans*. *Mycopathologia*. 1978; 65: 73–76.
46. Gullo FP, Rossi SA, Sardi JdeC, Teodoro VLI, Mendes-Giannini MJS, Fusco-Almeida AM. Cryptococcosis: epidemiology, fungal resistance, and new alternatives for treatment. *Eur J Clin Microbiol Infect Dis*. 2013; 32: 1377–1391.
47. Ikeda R, Shinoda T, Fukazawa Y, Kaufman LEO. Antigenic characterization of *Cryptococcus neoformans* serotypes and its application to serotyping of clinical isolates. *J Clin Microbiol*. 1982; 16: 22–29.
48. Franzot SP, Salkin IF, Casadevall A. *Cryptococcus neoformans var. grubii*: Separate varietal status for *Cryptococcus neoformans* serotype A isolates. *J Clin Microbiol*. 1999; 37: 838–840.
49. Jarvis JN, Harrison TS. Pulmonary cryptococcosis. *Crit Care*. 2008; 1: 141–150.
50. Kwon-chung KJ, Boekhout T, Fell JW, Diaz M. Proposal to conserve the name *Cryptococcus gattii* against *C. hondurianus* and *C. bacillisporus* (Basidiomycota, Hymenomycetes, Tremellomycetidae). *Taxon*. 2002; 51: 804–806.
51. Kwon-chung KJ. A new genus, Filobasidiella, the perfect state of *Cryptococcus neoformans*. *Mycologia*. 1975; 67: 1197–1200.
52. Kwon-Chung KJ, Varma A. Do major species concepts support one, two or more species within *Cryptococcus neoformans*? *FEMS Yeast Res*. 2006; 6: 574–587.
53. Hagen F, Khayhan K, Theelen B et al. Recognition of seven species in the *Cryptococcus gattii* / *Cryptococcus neoformans* species complex. *Fungal Genet Biol*. 2015; 78: 16–48.
54. Dromer F, Mathoulin-Pélissier S, Launay O et al. Determinants of disease presentation and outcome during cryptococcosis: the CryptoA/D study. *PLoS Med*. 2007; 4: e21.
55. Barchiesi F, Cogliati M, Esposto MC et al. Comparative analysis of pathogenicity of *Cryptococcus neoformans* serotypes A, D and AD in murine cryptococcosis. *J Infect*. 2005; 51: 10–16.
56. Mitchell TG, Perfect JR. Cryptococcosis in the era of AIDS: 100 years after the discovery of *Cryptococcus neoformans*. *Clin Microbiol Rev*. 1995; 8: 515–548.
57. Meyer W, Aanensen DM, Boekhout T et al. Consensus multi-locus sequence typing scheme for *Cryptococcus neoformans* and *Cryptococcus gattii*. *Med Mycol*. 2009; 47: 561–570.
58. Litvintseva AP, Thakur R, Vilgalys R, Mitchell TG. Multilocus sequence typing reveals three genetic subpopulations of *Cryptococcus neoformans var. grubii (serotype* A), including a unique population in Botswana. *Genetics*. 2006; 172: 2223–2238.
59. Diaz MR, Boekhout T, Kiesling T, Fell JW. Comparative analysis of the intergenic spacer regions and population structure of the species complex of the pathogenic yeast *Cryptococcus neoformans*. *FEMS Yeast Res*. 2005; 5: 1129–1140.
60. Bovers M, Hagen F, Kuramae EE, Boekhout T. Six monophyletic lineages identified within *Cryptococcus neoformans* and *Cryptococcus gattii* by multi-locus sequence typing. *Fungal Genet Biol*. 2008; 45: 400–421.
61. Meyer W, Lieckfeldt E, Kuhls K, EZ F, Börner T, TG M. DNA and PCR fingerprinting in fungi. In: *DNA Fingerprinting: State of the Science*. *Berlin: Birkhäuser Basel*, 1993: 311–320.
62. Spitzer ED, Spitzer SG. Use of a dispersed repetitive DNA element to distinguish clinical isolates of *Cryptococcus* use of a dispersed repetitive DNA element to distinguish clinical isolates of *Cryptococcus neoformans*. *J Clin Microbiol*. 1992; 30: 1094–1097.
63. Litvintseva AP, Mitchell TG. Most environmental isolates of *Cryptococcus neoformans var. grubii* (serotype A) are not lethal for mice. *Infect Immun*. 2009; 77: 3188–3195.
64. Beale MA, Sabiiti W, Robertson EJ et al. Genotypic diversity is associated with clinical outcome and phenotype in cryptococcal meningitis across Southern Africa. *PLoS Negl Trop Dis*. 2015; 9: 1–18.
65. Wiesner DL, Moskalenko O, Corcoran JM et al. Cryptococcal genotype influences immunologic response and human clinical outcome after meningitis. *MBio*. 2012; 3: 1–10.
66. Khayhan K, Hagen F, Pan W et al. Geographically structured populations of *Cryptococcus neoformans variety grubii* in Asia correlate with HIV status and show a clonal population structure. *PLoS One*. 2013; 8: 1–14.
67. Meyer W, Castaneda A, Jackson S, Huynh M, Castaneda E. the IberoAmerican Cryptococcal Study Group. Molecular typing of IberoAmerican *Cryptococcus neoformans* isolates. *Emerg Infect Dis*. 2003; 9: 189–195.
68. Ngamskulrungroj P, Chang Y, Roh J, Kwon-Chung KJ. Differences in nitrogen metabolism between *Cryptococcus neoformans* and *C. gattii*, the two etiologic agents of cryptococcosis. *PLoS One*. 2012; 7.
69. Firacative C, Trilles L, Meyer W. MALDI-TOF MS enables the rapid identification of the major molecular types within the *Cryptococcus neoformans /C. gattii* species complex. *PLoS One*. 2012; 7.
70. Okubo Y, Wakayama M, Ohno H et al. Histopathological study of murine pulmonary cryptococcosis induced by *Cryptococcus neoformans* and *Cryptococcus gattii*. *Jpn J Infect Dis*. 2013; 66: 216–221.
71. Ngamskulrungroj P, Chang Y, Sionov E, Kwon-chung KJ. The primary target organ of *Cryptococcus gattii* is different from that of *Cryptococcus neoformans* in a murine model. *mBio Am Soc Microbiol*. 2012; 3: 1–9.
72. Kwon-Chung KJ, Bennett JE, Wickes BL et al. The case for adopting the "species complex" nomenclature for the etiologic agents of cryptococcosis. *Msph Am Soc Microbiol*. 2017; 2: e00357–16.
73. Pfaller M, Diekema D, Gibbs D et al. Results from the ARTEMIS DISK global antifungal surveillance study, 1997 to 2007: 10.5-year analysis of susceptibilities of noncandidal yeast species to fluconazole and voriconazole determined by CLSI standardized disk diffusion testing. *J Clin Microbiol*. 2009; 47: 117–123.
74. De Almeida GMD, Costa SF, Melhem M et al. *Rhodotorula* spp. isolated from blood cultures: clinical and microbiological aspects. *Med Mycol*. 2008; 46: 547–556.
75. 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: 17258–17263.
76. Dixit A, Carroll SF, Qureshi ST. *Cryptococcus gattii*: an emerging cause of fungal disease in North America. *Interdiscip Perspect Infect Dis*. 2009; 2009: 840452.
77. Harris J, Lockhart S, Chiller T. *Cryptococcus gattii*: where do we go from here? *Med Mycol*. 2012; 50: 113–129.

78. Okamoto K, Hatakeyama S, Itoyama S et al. *Cryptococcus gattii* genotype VGIIa infection in man, Japan, 2007. *Emerg Infect Dis*. 2010; 16: 1155–1157.
79. Kitaura T, Takahashi M, Umeyama T et al. *Cryptococcus gattii* genotype VGIIa infection imported from Vancouver Island to Japan. *J Infect Chemother*. 2018; 24: 573–575.
80. Cogliati M. Global molecular epidemiology of *Cryptococcus neoformans* and *Cryptococcus gattii*: an atlas of the molecular types. *Scientifica (Cairo)*. 2013; 2013: 657213.
81. Liaw SJ, Wu HC, Hsueh PR. Microbiological characteristics of clinical isolates of *Cryptococcus neoformans* in Taiwan: serotypes, mating types, molecular types, virulence factors, and antifungal susceptibility. *Clin Microbiol Infect*. 2010; 16: 696–703.
82. Fang W, Fa Z, Liao W. Epidemiology of *Cryptococcus* and cryptococcosis in China. *Fungal Genet Biol*. 2015; 78: 7–15.
83. Viviani MA, Cogliati M, Esposto MC et al. Molecular analysis of 311 *Cryptococcus neoformans* isolates from a 30-month ECMM survey of cryptococcosis in Europe. *FEMS Yeast Res*. 2006; 6: 614–619.
84. Antinori S. New insights into HIV / AIDS-associated cryptococcosis. *ISRN AIDS*. 2013; 2013: 471363.
85. Pan W, Khayhan K, Hagen F et al. Resistance of Asian *Cryptococcus neoformans* serotype A is confined to few microsatellite genotypes. *PLoS One*. 2012; 7: e32868.
86. Shirley RM, Baddley JW. Cryptococcal lung disease. *Curr Opin Pulm Med*. 2009; 15: 254–260.
87. Yamamoto Y, Kohno S, Koga H et al. Random amplified polymorphic DNA analysis of clinically and environmentally isolated *Cryptococcus neoformans* in Nagasaki. *J Clin Microbiol*. 1995; 33: 3328–3332.
88. Sorrell TC, Chen SCA, Ruma P et al. Concordance of clinical and environmental isolates of *Cryptococcus neoformans var. gattii* by random amplification of polymorphic DNA analysis and PCR fingerprinting. *J Clin Microbiol*. 1996; 34: 1253–1260.
89. Velagapudi R, Hsueh YP, Geunes-Boyer S, Wright JR, Heitman J. Spores as infectious propagules of *Cryptococcus neoformans*. *Infect Immun*. 2009; 77: 4345–4355.
90. Botts MR, Hull CM. Dueling in the lung: How *Cryptococcus* spores race the host for survival. *Curr Opin Microbiol*. 2010; 13: 437–442.
91. Mody CH, Warren PW. Host defence to pulmonary mycosis. *Can J Infect Dis*. 1999; 10: 147–155.
92. Zimmer B, Hempel H, Goodman N. Pathogenicity o f the basidiospores of *Filobasidiella neoformans*. *Mycopathologia*. 1984; 85: 149–153.
93. Gibson JF, Johnston SA. Immunity to *Cryptococcus neoformans* and *C. gattii* during cryptococcosis. *Fungal Genet Biol*. 2015; 78: 76–86.
94. Wozniak KL, Vyas JM, Levitz SM. In vivo role of dendritic cells in a murine model of pulmonary cryptococcosis. *Infect Immun*. 2006; 74: 3817–3824.
95. Holmer SM, Evans KS, Asfaw YG et al. Impact of surfactant protein D, interleukin-5, and eosinophilia on cryptococcosis. *Infect Immun*. 2014; 82: 683–693.
96. Garro AP, Chiapello LS, Baronetti JL, Masih DT. Rat eosinophils stimulate the expansion of *Cryptococcus neoformans*-specific CD4+ and CD8+ T cells with a T-helper 1 profile. *Immunology*. 2011; 132: 174–187.
97. Guillot L, Carroll SF, Homer R, Qureshi ST. Enhanced innate immune responsiveness to pulmonary *Cryptococcus neoformans* infection is associated with resistance to progressive infection. *Infect Immun*. 2008; 76: 4745–4756.
98. McQuiston T, Luberto C, Poeta MDel. Role of sphingosine-1-phosphate (S1P) and S1P receptor 2 in the phagocytosis of *Cryptococcus neoformans* by alveolar macrophages. *Microbiology*. 2011; 157: 1416–1427.
99. Baker RD. The primary pulmonary lymph node complex of cryptococcosis. *Am J Clin Pathol*. 1976; 65: 83–92.
100. Salyer WR, Salyer DC, Baker RD. Primary complex of *Cryptococcus* and pulmonary lymph nodes. *J Infect Dis*. 1974; 130: 74–77.
101. 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: 2229–2239.
102. Sweeney DA, Caserta MT, Korones DN, Casadevall A, Goldman DL. A ten-year-old boy with a pulmonary nodule secondary to *Cryptococcus neoformans*: case report and review of the literature. *Pediatr Infect Dis J*. 2003; 22: 1089–1093.
103. Levitz SM, Dupont MP, Smail EH. Direct activity of human T lymphocytes and natural killer cells against *Cryptococcus neoformans*. *Infect Immun*. 1994; 62: 194–202.
104. Chun FZ, Ling LM, Jones GJ et al. Cytotoxic CD4+ T cells use granulysin to kill *Cryptococcus neoformans*, and activation of this pathway is defective in HIV patients. *Blood J Am Soc Hematol*. 2007; 109: 2049–2057.
105. Goldman DL, Davis J, Bommarito F, Shao X, Casadevall A. Enhanced allergic inflammation and airway responsiveness in rats with chronic *Cryptococcus neoformans* infection: potential role for fungal pulmonary infection in the pathogenesis of asthma. *J Infect Dis*. 2006; 193: 1178–1186.
106. Grahnert A, Muller U, Von Buttlar H, Treudler R, Alber G. Analysis of asthma patients for cryptococcal seroreactivity in an urban German area. *Med Mycol*. 2015; 53: 576–586.
107. Eisenman HC, Casadevall A, McClelland EE. New insights on the pathogenesis of invasive *Cryptococcus neoformans* infection. *Curr Infect Dis Rep*. 2007; 9: 457–464.
108. Heitman J, Kozel T, Kwon-Chung KJ, Perfect JR, Casadevall A, eds. *Cryptococcus: From Human Pathogen to Model Yeast*. Vol 11. Washington, DC: ASM Press, 2011.
109. Cecília Bittencourt Severo, Alexandra Flávia Gazzoni LCS. Pulmonary cryptococcosis. *J Bras Pneumol*. 2009; 35: 1136–1144.
110. Zaragoza O, Rodrigues ML, Jesus MDe et al. The capsule of the fungal pathogen *Cryptococcus neoformans*. *Adv Appl Microbiol*. 2009; 68: 1–64.
111. Nosanchuk JD, Casadevall A. Impact of melanin on microbial virulence and clinical resistance to antimicrobial compounds. *Antimicrob Agents Chemother*. 2006; 50: 3519–3528.
112. Perfect JR. *Cryptococcus neoformans*: The yeast that likes it hot. *FEMS Yeast Res*. 2006; 6: 463–468.
113. Rodrigues ML, Nakayasu ES, Oliveira DL et al. Extracellular vesicles produced by *Cryptococcus neoformans* contain protein components associated with virulence. *Eukaryot Cell*. 2008; 7: 58–67.
114. Levitz SM, Specht CA. The molecular basis for the immunogenicity of *Cryptococcus neoformans* mannoproteins. *FEMS Yeast Res*. 2006; 6: 513–524.
115. Vecchiarelli A, Pietrella D, Dottorini M et al. Encapsulation of *Cryptococcus neoformans* regulates fungicidal activity and the antigen presentation process in human alveolar macrophages. *Clin Exp Immunol*. 1994; 98: 217–223.
116. Jacobson ES, Tinnell SB. Antioxidant function of fungal melanin. *J Bacteriol*. 1993; 175: 7102–7104.
117. Giles SS, Batinic-Haberle I, Perfect JR, Cox GM. *Cryptococcus neoformans* mitochondrial superoxide dismutase: an essential link between antioxidant function and high-temperature growth. *Eukaryot Cell*. 2005; 4: 46–54.
118. Voelz K, Johnston SA, Smith LM, Hall RA, Idnurm A, May RC. "Division of labour" in response to host oxidative burst drives a fatal *Cryptococcus gattii* outbreak. *Nat Commun*. 2014; 5: 5194.
119. Okagaki LH, Nielsen K. Titan cells confer protection from phagocytosis in *Cryptococcus neoformans* infections. *Eukaryot Cell*. 2012; 11: 820–826.
120. Feldmesser M, Kress Y, Casadevall A. Dynamic changes in the morphology of *Cryptococcus neoformans* during murine pulmonary infection. *Microbiology*. 2001; 147: 2355–2365.
121. Zaragoza O, Nielsen K. Titan cells in *Cryptococcus neoformans*: cells with a giant impact. *Curr Opin Microbiol*. 2013; 16: 409–413.
122. Zaragoza O, Rocio G-R, Nosanchuk JD, Cuenca-Estrella M, Rodriguez-Tudela JL, Casadevall A. Fungal cell gigantism during mammalian infection. *PLoS Pathog*. 2010; 6: e1000945.
123. Crabtree JN, Okagaki LH, Wiesner DL, Strain AK, Nielsen JN, Nielsen K. Titan cell production enhances the virulence of *Cryptococcus neoformans*. *Infect Immun*. 2012; 80: 3776–3785.
124. Ngamskulrungroj P, Price J, Sorrell T, Perfect JR, Meyer W. *Cryptococcus gattii* virulence composite: Candidate genes revealed by microarray analysis of high and less virulent Vancouver Island outbreak strains. *PLoS One*. 2011; 6: e16076.
125. Liu L, Tewari RP, Williamson PR. Laccase protects *Cryptococcus neoformans* from antifungal activity of alveolar macrophages. *Infect Immun*. 1999; 67: 6034–6039.
126. Eisenman HC, Mues M, Weber SE et al. *Cryptococcus neoformans* laccase catalyses melanin synthesis from both D- and L-DOPA. *Microbiology*. 2007; 153: 3954–3962.

127. Erb-Downward JR, Noggle RM, Williamson PR, Huffnagle GB. The role of laccase in prostaglandin production by *Cryptococcus neoformans*. *Mol Microbiol*. 2008; 68: 1428–1437.
128. Qiu Y, Davis MJ, Dayrit JK et al. Immune modulation mediated by cryptococcal laccase promotes pulmonary growth and brain dissemination of virulent *Cryptococcus neoformans* in mice. *PLoS One*. 2012; 7: e47853.
129. Huang J, Lan C, Li H, Lin Q, Liu J, Wen H. Retrospective analysis of 117 chinese patients with pulmonary cryptococcosis. *Chest*. 2016; 149:A124.
130. Chen SCA, Meyer W, Sorrell TC. *Cryptococcus gattii* infections. *Clin Microbiol Rev*. 2014; 27: 980–1024.
131. Nakao M, Muramatsu H, Takahashi T et al. *Cryptococcus gattii* genotype VGIIa infection in an immunocompetent Japanese patient: a case report and mini-review. *Intern Med*. 2016; 55: 3021–3024.
132. Zhang B, Cao W, Wu X, Wang S. Pulmonary cryptococcosis in an immunocompetent patient: a case report. *Int J Clin Exp Pathol*. 2017; 10: 9798–9801.
133. Kanjanapradit K, Kosjerina Z, Tanomkiat W, Keeratichananont W, Puntuwong S. Pulmonary cryptococcosis presenting with lung mass: report of 7 cases and review of literature. *Clin Med Insights Pathol*. 2017; 10: 1–5.
134. Ruan Q, Zhu Y, Chen S, Zhu L, Zhang S, Zhang W. Disseminated cryptococcosis with recurrent multiple abscesses in an immunocompetent patient: a case report and literature review. *BMC Infect Dis*. 2017; 17: 369.
135. Kohno S, Kakeya H, Izumikawa K et al. Clinical features of pulmonary cryptococcosis in non-HIV patients in Japan. *J Infect Chemother*. 2015; 21: 23–30.
136. Xie X, Xu B, Yu C et al. Clinical analysis of pulmonary cryptococcosis in non-HIV patients in south China. *Int J Clin Exp Med*. 2015; 8: 3114–3119.
137. Kiertiburanakul S, Wirojtananugoon S, Pracharktam R, Sungkanuparph S. Cryptococcosis in human immunodeficiency virus-negative patients. *Int J Infect Dis*. 2006; 10: 72–78.
138. Jain S, Mahajan V, Kumar A. Unusual case of coexistent pulmonary cryptococcosis and tuberculosis in an immuno-competent host. *Indian J Tuberc*. 2017; 64: 228–231.
139. Sawai T, Nakao T, Koga S et al. Miliary tuberculosis with co-existing pulmonary cryptococcosis in non-HIV patient without underlying diseases: a case report. *BMC Pulm Med*. 2018; 18: 1–5.
140. Thomas R, Christopher D, Balamugesh T, James P, Thomas M. Coexisting pulmonary cryptococcosis and pulmonary tuberculosis in an immunocompetent host. *Singapore Med J*. 2012; 53: 32–34.
141. Huang CL, Chen CT, Wu SW, Lin TY. Simultaneous coinfection with *Cryptococcus liquefaciens* and *Mycobacterium tuberculosis* in an adult. *Q J Med*. 2014; 107: 223–224.
142. Nabaei G, Afhami S. Disseminated cryptococcosis and active pulmonary tuberculosis co- infection in an otherwise healthy adult. 2015; 14: 174–176.
143. Chomicki J. Coexistence of pulmonary tuberculosis with pulmonary and meningeal cryptococcosis. *Dis Chest*. 1966; 50: 214–216.
144. Fang W, Zhang L, Liu J et al. Tuberculosis/cryptococcosis co-infection in China between 1965 and 2016. *Emerg Microbes Infect*. 2017; 6: e73.
145. Musabende M, Mukabatsinda C, Riviello ED, Ogbuagu O. Concurrent cryptococcal meningitis and disseminated tuberculosis occurring in an immunocompetent male. *BMJ Case Rep*. 2016; pii: bcr2015213380.
146. Hu X, Wang R, Wang X et al. Dectin-2 polymorphism associated with pulmonary cryptococcosis in HIV-uninfected Chinese patients. *Med Mycol*. 2015; 53: 810–816.
147. Hajjeh RA, Conn LA, Stephens DS et al. Cryptococcosis: population-based multistate active surveillance and risk factors in human immunodeficiency virus – infected persons. *J Infect Dis*. 1999; 179: 449–454.
148. Pappas PG. Cryptococcal infections in non-HIV-infected patients. *Trans Am Clin Climatol Assoc*. 2013; 124: 61–79.
149. Yu J-Q, Tang K-J, Xu B-L, Xie C-M, Light RW. Pulmonary cryptococcosis in non-AIDS patients. *Braz J Infect Dis*. 2012; 16: 531–539.
150. Wong ML, Back P, Candy G, Nelson G, Murray J. Cryptococcal pneumonia in African miners at autopsy. *Int J Tuberc Lung Dis*. 2007; 11: 528–533.
151. Houston S. Who determines the "standard of care" in research in resource poor countries? *Int J Tuberc Lung Dis*. 2009; 13: 149.
152. Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *AIDS*. 2009; 23: 525–530.
153. Lanjewar DN, Duggal R. Pulmonary pathology in patients with AIDS: An autopsy study from Mumbai. *HIV Med*. 2001; 2: 266–271.
154. Bernicker EH, Atmar RL, Schaffner DL, Greenberg SB. Unanticipated diagnoses found at autopsy in an urban public teaching hospital. *Am J Med Sci*. 1996; 311: 215–220.
155. Kaur R, Mehra B, Dhakad MS, Goyal R, Bhalla P, Dewan R. Fungal opportunistic pneumonias in HIV/AIDS patients: an Indian tertiary care experience. *J Clin Diagnostic Res*. 2017; 11: DC14–DC19.
156. Pappas PG, Alexander BD, Andes DR et al. Invasive fungal infections among organ transplant recipients: results of the transplant-associated infection surveillance network (TRANSNET). *Clin Infect Dis*. 2010; 50: 1101–1111.
157. Ponzio V, Fernando L, José C, Robert J, Arnaldo P, Colombo L. Outcomes of cryptococcosis in renal transplant recipients in a resourced health care system. *Transpl Infect Dis*. 2018; 20: e12910.
158. Singh N, Alexander BD, Lortholary O et al. *Cryptococcus neoformans* in organ transplant recipients: Impact of calcineurin-inhibitor agents on mortality. *J Infect Dis*. 2007; 195: 756–764.
159. George I, Santos C, Powderly W, Olsen M. Epidemiology of cryptococcosis and cryptococcal meningitis in a large retrospective cohort of patients following solid organ transplantation. *Open Forum Infect Dis*. 2017; 4: ofx004.
160. Sun H, Wagener MM, Singh N. Cryptococcosis in solid-organ, hematopoietic stem cell, and tissue transplant recipients: Evidence-based evolving trends. *Clin Infect Dis*. 2009; 48: 1566–1576.
161. Kuroda A, Tasaka S, Yagi K et al. A case of disseminated cryptococcal infection and concurrent lung tuberculosis in a patient under steroid therapy for interstitial pneumonia. *Case Rep Pulmonol*. 2015; 2015: 358926.
162. Yuri T, Kimura A, Yoshizawa K, Emoto Y, Kinoshita Y, Tsubura A. Pulmonary and meningeal cryptococcosis after corticosteroid therapy for autoimmune hepatitis: Coexistence of cryptococci within pulmonary cancer nodule. *Case Rep Pathol*. 2013; 2013: 807197.
163. Schmalzle SA, Buchwald UK, Gilliam BL, Riedel DJ. *Cryptococcus neoformans* infection in malignancy. *Mycoses*. 2016; 59: 542–552.
164. Wang R, Chen Y, Wu J et al. Cryptococcosis in patients with hematological diseases: a 14-year retrospective clinical analysis in a Chinese tertiary hospital. *BMC Infect Dis*. 2017; 17: 463.
165. Prasad KT, Sehgal IS, Shivaprakash MR, Dhooria S. Uncommon mycosis in a patient with diabetes. *BMJ Case Rep*. 2016; 2016: pii: bcr2016214453.
166. Li Y, Fang W, Jiang W et al. Cryptococcosis in patients with diabetes mellitus II in mainland China: 1993–2015. *Mycoses*. 2017; 60: 706–713.
167. Lin K-H, Chen C-M, Chen T-L et al. Diabetes mellitus is associated with acquisition and increased mortality in HIV-uninfected patients with cryptococcosis: a population-based study. *J Infect*. 2016; 72: 608–614.
168. Vilchez RA, Linden P, Lacomis J, Costello P. Acute respiratory failure associated with pulmonary cryptococcosis in non- AIDS patients. *Chest*. 2001; 119: 1865–1869.
169. Inaba A, Okada A, Yoshida T et al. Disseminated cryptococcosis with rapidly growing lung nodules in an end-stage renal disease patient. *Intern Med*. 2017; 56: 377–380.
170. Singh N, Husain S, De Vera M, Gayowski T, Cacciarelli T V. *Cryptococcus neoformans* infection in patients with cirrhosis, including liver transplant candidates. *Medicine (Baltimore)*. 2004; 83: 188–192.
171. Singh N, Sifri CD, Silveira FP et al. Cryptococcosis in patients with cirrhosis of the liver and posttransplant outcomes. *Transplantation*. 2015; 99: 2132–2141.
172. Spec A, Raval K, Powderly WG. End-stage liver disease is a strong predictor of early mortality in cryptococcosis. *Open Forum Infect Dis*. 2016; 3: 1–5.
173. Hokari S, Tsukada H, Ito K, Shibuya H. An autopsy case of disseminated cryptococcosis manifesting as acute diarrhea in a patient with primary biliary cirrhosis. *Intern Med*. 2010; 49: 1793–1796.
174. Vilchez RA, Irish W, Lacomis J, Costello P, Fung J, Kusne S. The clinical epidemiology of pulmonary cryptococcosis in non-AIDS patients at a tertiary care medical center. *Medicine (Baltimore)*. 2001; 80: 308–312.
175. 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.

176. Lewis JL, Rabinovich S. The wide spectrum of cryptococcal infections. *Am J Med*. 1972; 53: 315–322.
177. Behrman RE, Masci JR, Nicholas P. Cryptococcal skeletal infections: Case report and review. *Rev Infect Dis*. 1990; 12: 181–190.
178. Silveira FP, Husain S, Kwak EJ et al. Cryptococcosis in liver and kidney transplant recipients receiving anti-thymocyte globulin or alemtuzumab. *Transpl Infect Dis*. 2007; 9: 22–27.
179. Aberg JA, Mundy LM, Powderly WG. Pulmonary cryptococcosis in patients without HIV infection. *CHEST J*. 1999; 115: 734–740.
180. Pappas PG, Perfect JR, Cloud Ga et al. Cryptococcosis in human immunodeficiency virus-negative patients in the era of effective azole therapy. *Clin Infect Dis*. 2001; 33: 690–699.
181. Smith KD, Achan B, Hullsiek KH et al. Increased antifungal drug resistance in clinical isolates of *Cryptococcus neoformans* in Uganda. *Antimicrob Agents Chemother*. 2015; 59: 7197–7204.
182. Wei-Chou Chang, Ching Tzao, Hsian-He Hsu et al. Pulmonary cryptococcosis * Comparison of clinical and radiographic characteristics in immunocompetent and immunocompromised patients. *Chest*. 2006; 129: 333–340.
183. Feliciano LG, Deseda C, Rivera A et al. Emergence of *Cryptococcus gattii* - Pacific Northwest, 2004–2010. *Morb Mortal Wkly Report, CDC*. 2010; 59: 865–893.
184. Cameron ML, Bartlett JA, Gallis HA, Waskin HA. Manifestations of pulmonary cryptococcusis in patients with acquired immunodeficiency syndrome. *Clin Infect Dis*. 1990; 13: 64–67.
185. Chechani V, Kamholz SL. Pulmonary manifestations of disseminated cryptococcosis in patients with AIDS. *Chest*. 1990; 98: 1060–1066.
186. Gunda DW, Bakshi FA, Rambau P, Kilonzo SB. Pulmonary cryptococcosis presenting as acute severe respiratory distress in a newly diagnosed HIV patient in Tanzania: a case report. *Clin Case Reports*. 2015; 3: 749–752.
187. Scriven JE, Botha FC, Schutz C, Lalloo DG, Wainwright H, Meintjes G. Paradoxical respiratory failure due to cryptococcal pneumonia after amphotericin B treatment for HIV-associated cryptococcal meningitis. *Med Mycol Case Rep*. 2018; 19: 38–40.
188. Liang Y, Shen Y, Zhang J, Zhong X. Pulmonary cryptococcosis among 3 immunocompetent patients misdiagnosed as bacterial pneumonia and pulmonary tuberculosis. *Chest*. 2016; 149: 114A.
189. Riha RL, Pataka AD. BMJ best practice: cryptococcosis. *Br Med Journals*. 2014.
190. Miller KD, Mican JA, Davey RT. Asymptomatic solitary pulmonary nodules due to *Cryptococcus neoformans* in patients infected with human immunodeficiency virus. *Clin Infect Dis*. 1996; 23: 810–812.
191. Chen SCA, Meyer W, Sorrell TC. *Cryptococcus gattii* infections. *Clin Microbiol Rev*. 2014; 27: 980–1024.
192. Guimaraes MD, Marchiori E, De Souza Portes Meirelles G et al. Fungal infection mimicking pulmonary malignancy: Clinical and radiological characteristics. *Lung*. 2013; 191: 655–662.
193. Choi HW, Chong S, Kim MK, Park IW. Pulmonary cryptococcosis manifesting as diffuse air-space consolidations in an immunocompetent patient. *J Thorac Dis*. 2017; 9: E138–E141.
194. Ang SYL, Ng VWL, Kumar SD, Low SYY. Cryptococcosis mimicking lung carcinoma with brain metastases in an immunocompetent patient. *J Clin Neurosci*. 2016; 35: 10–12.
195. Zheng S, Tan TT, Chien JMF. *Cryptococcus gattii* infection presenting as an aggressive lung mass. *Mycopathologia*. 2018; 183: 597–602.
196. Godwin JD, Ravin CE, Gallis HA, Putman E. Thoracic cryptococcosis: Immunologic competence and radiologic appearance. *Am J Roentgenol*. 1984; 141: 893–896.
197. Si-yun W, Gang C, Dong-lan L et al. F-FDG PET / CT and contrast-enhanced CT findings of pulmonary cryptococcosis. *Eur J Radiol*. 2017; 89: 140–148.
198. Basnayake TL, Lim A, Currie BJ. Pulmonary cryptococcal infection presenting with multiple lung nodules. *Respir Med Case Reports*. 2018; 23: 122–124.
199. de Farias LdePG, Padilha IG, Lemos MRL, Dos Santos CJJ, de Miranda CMN. Pulmonary cryptococcosis mimicking neoplasm in trems of uptake PET/CT. *Radiol Bras*. 2018; 51: 63–64.
200. Kelly S, Marriott D. Miliary pulmonary cryptococcosis. *Med Mycol Case Rep*. 2014; 6: 22–24.
201. Hu Z, Chen J, Wang J et al. Radiological characteristics of pulmonary cryptococcosis in HIV-infected patients. *PLoS One*. 2017; 12: 1–9.
202. Lindell RM, Hartman TE, Nadrous HF, Ryu JH. Pulmonary cryptococcosis: CT findings in immunocompetent patients. *Radiology*. 2005; 236: 326–331.
203. Choe YH, Moon H, Park SJ et al. Pulmonary cryptococcosis in asymptomatic immunocompetent hosts. *Scand J Infect Dis*. 2009; 41: 602–607.
204. Gazzoni AF, Severo CB, Salles EF, Severo LC. Histopathology, serology and cultures in the diagnosis of cryptococcosis. *Rev Inst Med Trop Sao Paulo*. 2009; 51: 255–259.
205. De Pauw B, Walsh TJ, Donnelly JP et al. Revised definitions of invasive fungal disease from the European Organization for Research & Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses (EORTC/MSG) Consensus Group. *Clin Infect Dis*. 2008; 46: 1813–1821.
206. Guarner J, Brandt ME. Histopathologic diagnosis of fungal infections in the 21st century. *Clin Microbiol Rev*. 2011; 24: 247–280.
207. Thibodeau KP, Viera AJ. Atypical pathogens and challenges in community-acquired pneumonia. *Am Acad Fam Physicians*. 2005; 47: 14–19.
208. 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.
209. Saha DC, Xess I, Biswas A, Bhowmik DM, Padma M V. Detection of cryptococcus by conventional, serological and molecular methods. *J Med Microbiol*. 2009; 58: 1098–1105.
210. José IG, Johnson M, Jurgen R, Schlomo S, Williams . British HIV Association and British Infection Association Guidelines for the Treatment of Opportunistic Infection in HIV-seropositive Individuals 2011. *HIV Med*. 2011; 12: 1–140.
211. WHO. Rapid advice: diagnosis, prevention and management of cryptococcal disease in *HIV-infected adults, adolescents and children: December 2011*. Geneva: World Heal Organ. 2011.
212. Gazzoni AF, Oliveira FdeM, Salles EF et al. Unusual morphologies of *Cryptococcus spp*. in tissue specimens: Report of 10 cases. *Rev Inst Med Trop Sao Paulo*. 2010; 52: 145–149.
213. Dromer F, Lortholary O. Cryptococcosis. *Hunter's Trop Med Emerg Infect Dis*. 2013: 641–643.
214. Perfect JR, Dismukes WE, Dromer F et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the infectious diseases society of america. *Clin Infect Dis*. 2010; 50: 291–322.
215. Jain N, Fries BC. Phenotypic switching of *Cryptococcus neoformans* and *Cryptococcus gattii*. *Mycopathologia*. 2008; 166: 181–188.
216. Jain N, Wickes BL, Keller SM et al. Molecular epidemiology of clinical *Cryptococcus neoformans* strains from India. *J Clin Microbiol*. 2005; 43: 5733–5742.
217. Denning DW, Stevens DA, Hamilton JR. Comparison of *Guizotia abyssinica* seed extract (birdseed) agar with conventional media for selective identification of *Cryptococcus neoformans* in patients with acquired immunodeficiency syndrome. *J Clin Microbiol*. 1990; 28: 2565–2567.
218. Pham CD, Ahn S, Turner LA, Wohrle R, Lockhart SR. Development and validation of benomyl birdseed agar for the isolation of *Cryptococcus neoformans* and *Cryptococcus gattii* from environmental samples. *Med Mycol*. 2014; 52: 417–421.
219. Diaz MR, Nguyen MH. Diagnostic approach based on capsular antigen, capsule detection, Beta-glucan, and DNA analysis. In: *Cryptococcus: From Human Pathogen to Model Yeast*. Washington, DC: ASM Press, 2011: 547–564.
220. Cheon WS, Eom K, Yoo BK, Kim D, Jung K. A case of pulmonary cryptococcosis by capsule-deficient *Cryptococcus neoformans*. *Korean J Intern Med*. 2006; 21: 83–87.
221. Ro JY, Lee SS, Ayala AG. Advantage of Fontana-Masson stain in capsule-deficient cryptococcal infection. *Arch Pathol Lab Med*. 1987; 111: 53–57.
222. Williamson JD, Silverman JF, Mallak CT, Christie JD. Atypical cytomorphologic appearance of *Cryptococcus neoformans*: a report of five cases. *Acta Cytol*. 1996; 40: 363–370.
223. Nava A, Orozco-Barocio G. Granulomatous lung disease an approach to the differential diagnosis. *Arch Pathol Lab Med*. 2010; 134: 667–690.
224. Jarvis JN, Wainwright H, Harrison TS, Rebe K, Meintjes G. Pulmonary cryptococcosis misdiagnosed as smear-negative pulmonary tuberculosis with fatal consequences. *Int J Infect Dis*. 2010; 14: e310–e312.
225. Shibuya K, Hirata A, Omuta J et al. Granuloma and cryptococcosis. *J Infect Chemother*. 2005; 11: 115–122.

226. Feldmesser M, Kress Y, Novikoff P. *Cryptococcus neoformans* is a facultative intracellular pathogen in murine pulmonary infection. *Infect Immun*. 2000; 68: 4225–4237.
227. Kawakami K, Kohno S, Murikawa N et al. Activation of macrophages and expansion of specific T lymphocytes in the lungs of mice intratracheally inoculated with *Cryptococcus neoformans*. *Clin Exp Immunol*. 1994; 96: 230–237.
228. 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: 1–6.
229. Pongsai P, Atamasirikul K, Sungkanuparph S. The role of serum cryptococcal antigen screening for the early diagnosis of cryptococcosis in HIV-infected patients with different ranges of CD4 cell counts. *J Infect*. 2010; 60: 474–477.
230. Baughman RP, Dohn MN, Henderson H, Frame PT. Detection of cryptococcal antigen in roncbhoalveolar lavage fluid: a prospective study of diagnostic utility. *Am Rev Respir Dis*. 1992; 145: 1226–1229.
231. Kralovic SM, Rhodes JC. Utility of routine testing of bronchoalveolar lavage fluid for cryptococcal antigen. *J Clin Microbiol*. 1998; 36: 3088–3089.
232. Liaw YS, Yang PC, Yu CJ et al. Direct determination of cryptococcal antigen in transthoracic needle aspirate for diagnosis of pulmonary cryptococcosis. *J Clin Microbiol*. 1995; 33: 1588–1591.
233. Senghor Y, Guitard J, Angoulvant A, Hennequin C. Cryptococcal antigen detection in broncho-alveolar lavage fluid. *Med Mycol*. 2018; 56: 774–777.
234. Oshima K, Takazono T, Saijo T et al. Examination of cryptococcal glucuronoxylomannan antigen in bronchoalveolar lavage fluid for diagnosing pulmonary cryptococcosis in HIV-negative patients. *Med Mycol*. 2018; 56: 88–94.
235. Jan IS, Cheng WC, Lo SC, Weng MH, Lee LN. *Cryptococcus neoformans* in sputum and lung aspiration cytology smears. *J Microbiol Immunol Infect*. 2014; 48: 463–464.
236. Gago S, Esteban C, Valero C, Zaragoza O, De La Bellacasa JP, Buitrago MJ. A multiplex real-time PCR assay for identification of *Pneumocystis jirovecii*, *Histoplasma capsulatum*,and *Cryptococcus neoformans*/*Cryptococcus gattii* in samples from AIDS patients with opportunistic pneumonia. *J Clin Microbiol*. 2014; 52: 1168–1176.
237. Feng X, Fu X, Ling B, Wang L, Liao W, Yao Z. Development of a singleplex PCR assay for rapid identification and differentiation of *Cryptococcus neoformans var. grubii, Cryptococcus neoformans var. neoformans, Cryptococcus gattii*, and hybrids. *J Clin Microbiol*. 2013; 51: 1920–1923.
238. Martins MdosA, Brighente KBS, de Matos TA, Vidal JE, de Hipolito DDC, Pereira-Chioccola VL. Molecular diagnosis of cryptococcal meningitis in cerebrospinal fluid: comparison of primer sets for *Cryptococcus neoformans* and *Cryptococcus gattii* species complex. *Brazilian J Infect Dis*. 2015; 19: 62–67.
239. Leal AL, Faganello J, Bassanesi MC, Vainstein MH. *Cryptococcus* species identification by multiplex PCR. *Med Mycol*. 2008; 46: 377–383.
240. Cordeiro RA, Costa AKF, Brilhante RSN et al. PCR-REA as an important tool for the identification of *Cryptococcus neoformans* and *Cryptococcus gattii* from human and veterinary sources. *Vet Microbiol*. 2011; 154: 180–184.
241. Esposto MC, Cogliati M, Tortorano AM, Viviani MA. Determination of *Cryptococcus neoformans var. neoformans* mating type by multiplex PCR. *Clin Microbiol Infect*. 2004; 10: 1092–1094.
242. Ogundeji AO, Albertyn J, Pohl CH, Sebolai OM. Method for identification of *Cryptococcus neoformans* and *Cryptococcus gattii* useful in resource-limited settings. *J Clin Pathol*. 2016; 69: 352–357.
243. Tanaka K, Miyazaki T, Maesaki S et al. Detection of *Cryptococcus neoformans* gene in patients with pulmonary crptococcosis. *J Clin Microbiol*. 1996; 34: 2826–2828.
244. Carvalho VG, Terceti MS, Dias ALT et al. Serotype and mating type characterization of *Cryptococcus neoformans* by multiplex PCR. *Rev Inst Med Trop Sao Paulo*. 2007; 49: 207–210.
245. Bialek R, Weiss M, Bekure-Nemariam K et al. Detection of *Cryptococcus neoformans* DNA in tissue samples by nested and real-time PCR assays. *Clin Diagn Lab Immunol*. 2002; 9: 461–469.
246. Rappelli P, Are R, Casu G, Fiori PL, Cappuccinelli P, Aceti A. Development of a nested PCR for detection of *Cryptococcus neoformans* cerebrospinal fluid. *J Clin Microbiol*. 1998; 36: 3438–3440.
247. Limper AH, Knox KS, Sarosi GA et al. An official American Thoracic Society statement: Treatment of fungal infections in adult pulmonary and critical care patients. *Am J Respir Crit Care Med*. 2011; 183: 96–128.
248. Molloy SF, Kanyama C, Heyderman RS et al. Antifungal combinations for treatment of cryptococcal meningitis in Africa. *N Engl J Med*. 2018; 378: 1004–1017.
249. Dromer F, Mathoulin S, Dupont B, Brugiere O, Letenneur L. Comparison of the efficacy of amphotericin B and fluconazole in the treatment of cryptococcosis in human immunodeficiency virus-negative patients: retrospective analysis of 83 cases. French Cryptococcosis Study Group. *Clin Infect Dis*. 1996; 22: S154–S160.
250. Nadrous HF, Antonius VS, Terrell CL, Ryu JH. Pulmonary cryptococcosis in nonimmunocompromised patients. *Chest*. 2003; 124: 2143–2147.
251. Bongomin F, Oladele RO, Gago S, Moore CB, Richardson MD. A systematic review of fluconazole resistance in clinical isolates of *Cryptococcus* species. *Mycoses*. 2018; 61: 290–297.
252. Chen YC, Chang TY, Liu JW et al. Increasing trend of fluconazole-non-susceptible *Cryptococcus neoformans* in patients with invasive cryptococcosis: a 12-year longitudinal study. *BMC Infect Dis*. 2015; 15: 277.
253. Geddes-McAlister J, Shapiro RS. New pathogens, new tricks: emerging, drug-resistant fungal pathogens and future prospects for antifungal therapeutics. *Ann N Y Acad Sci*. 2018: 1–22.
254. Shaw KJ, Schell WA, Covel J et al. In vitro and in vivo evaluation of APX001A/APX001 and other Gwt1 inhibitors against *Cryptococcus*. *Antimicrob Agents Chemother*. 2018; 62: pii: e00523-18.
255. Nishikawa H, Fukuda Y, Mitsuyama J et al. In vitro and in vivo antifungal activities of T-2307, a novel arylamidine, against *Cryptococcus gattii*: an emerging fungal pathogen. *J Antimicrob Chemother*. 2017; 72: 1709–1713.
256. Nielsen K, Vedula P, Smith KD et al. Activity of VT-1129 against *Cryptococcus neoformans* clinical isolates with high fluconazole MICs. *Med Mycol*. 2017; 55: 453–456.
257. Perfect JR. The antifungal pipeline: A reality check. *Nat Rev Drug Discov*. 2017; 16: 603–616.
258. Butts A, Didone L, Koselny K et al. A repurposing approach identifies off-patent drugs with fungicidal cryptococcal activity, a common structural chemotype, and pharmacological properties relevant to the treatment of cryptococcosis. *Eukaryot Cell*. 2013; 12: 278–287.
259. Lee KH, Chang UI, Kim HW et al. Acute respiratory failure associated with cryptococcal pneumonia and disseminated cryptococcosis in an AIDS patient. *Korean J Intern Med*. 2006; 21: 39–42.
260. Visnegarwala F, Graviss EA, Lacke CE et al. Acute respiratory failure associated with cryptococcosis in patients with AIDS: analysis of predictive factors. *Clin Infect Dis*. 1998; 27: 1231–1237.
261. Meyohas MC, Roux P, Bollens D et al. Pulmonary cryptococcosis: localized and disseminated infections in 27 patients with AIDS. *Clin Infect Dis*. 1995; 21: 628–633.
262. Parkes-Ratanshi R, Wakeham K, Levin J et al. Primary prophylaxis of cryptococcal disease with fluconazole in HIV-positive Ugandan adults: a double-blind, randomised, placebo-controlled trial. *Lancet Infect Dis*. 2011; 11: 933–941.
263. McKinsey DS, Wheat LJ, Cloud GA et al. Itraconazole prophylaxis for fungal infections in patients with advanced human immunodeficiency virus infection: randomized, placebo-controlled, double-blind study. National Institute of Allergy and Infectious Diseases Mycoses Study Group. *Clin Infect Dis*. 1999; 28: 1049–1056.
264. Powderly WG, Finkelstein DM, Feinberg J et al. A randomized trial comparing fluconazole with clotrimazole troches for the prevention of fungal infections in patients with advanced human immunodeficiency virus infection. *N Engl J Med*. 1995; 332: 700–705.
265. Hole CR, Wormley FL. Vaccine and immunotherapeutic approaches for the prevention of cryptococcosis: lessons learned from animal models. *Front Microbiol*. 2012; 3: 291.
266. Leopold Wager CM, Wormley FL. Is development of a vaccine against *Cryptococcus neoformans* feasible? *PLOS Pathog*. 2015; 11: e1004843.
267. Datta K, Lees A, Pirofski LA. Therapeutic efficacy of a conjugate vaccine containing a peptide mimotope of cryptococcal capsular

polysaccharide glucuronoxylomannan. *Clin Vaccine Immunol*. 2008; 15: 1176–1187.
268. Specht CA, Lee CK, Huang H et al. Protection against experimental cryptococcosis following vaccination with glucan particles containing cryptococcus alkaline extracts. *MBio*. 2015; 6: e01905–15.
269. Srichatrapimuk S, Sungkanuparph S. Integrated therapy for HIV and cryptococcosis. *AIDS Res Ther*. 2016; 13: 1–15.
270. Perfect JR, Bicanic T. Cryptococcosis diagnosis and treatment: What do we know now. *Fungal Genet Biol*. 2015; 78: 49–54.
271. Wormley FL, Perfect JR, Steele C, Cox GM. Protection against cryptococcosis by using a murine gamma interferon-producing *Cryptococcus neoformans* strain. *Infect Immun*. 2007; 75: 1453–1462.
272. Devi SJN, Schneerson R, Egan W et al. *Cryptococcus neoformans* serotype A glucuronoxylomannan-protein conjugate vaccines: synthesis, characterization, and immunogenicity. *Infect Immun*. 1991; 59: 3700–3707.
273. Fleuridor R, Lees A, Pirofski L. A cryptococcal capsular polysaccharide mimotope prolongs the survival of mice with *Cryptococcus neoformans* infection. *J Immunol*. 2001; 166: 1087–1096.
274. Duperval R, Hermans PE, Brewer NS, Roberts GD. Cryptococcosis, with emphasis on the significance of isolation of *Cryptococcus neoformans* from the respiratory tract. *Chest*. 1977; 72: 13–19.
275. Chechani V, Kamholz SL. Pulmonary manifestations of disseminated cryptococcosis in patients with AIDS. *Chest*. 1990; 98: 1061–1066.