INVITED REVIEW SERIES:
TRANSLATING RESEARCH INTO PRACTICE
SERIES EDITORS: JOHN E HEFFNER AND DAVID CL LAM

Pulmonary fungal infections

JEANNINA A. SMITH[1] AND CAROL A. KAUFFMAN[1,2]

[1]Division of Infectious Diseases, University of Michigan Medical School, and [2]Veterans Affairs Ann Arbor Healthcare System, Ann Arbor, Michigan, USA

ABSTRACT

This review details some of the advances that have been made in the recent decade in the diagnosis, treatment and epidemiology of pulmonary fungal infections. These advances have occurred because of increasing knowledge regarding the fungal genome, better understanding of the structures of the fungal cell wall and cell membrane and the use of molecular epidemiological techniques. The clinical implications of these advances are more rapid diagnosis and more effective and less toxic antifungal agents. For example, the diagnosis of invasive pulmonary aspergillosis, as well as histoplasmosis and blastomycosis, has improved with the use of easily performed antigen detection systems in serum and bronchoalveolar lavage fluid. Treatment of angioinvasive moulds has improved with the introduction of the new azoles, voriconazole and posaconazole that have broad antifungal activity. Amphotericin B is less frequently used, and when used is often given as lipid formulation to decrease toxicity. The newest agents, the echinocandins, are especially safe as they interfere with the metabolism of the fungal cell wall, a structure not shared with humans cells. Epidemiological advances include the description of the emergence of *Cryptococcus gattii* in North America and the increase in pulmonary mucormycosis and pneumonia due to *Fusarium* and *Scedosporium* species in transplant recipients and patients with haematological malignancies. The emergence of azole resistance among *Aspergillus* species is especially worrisome and is likely related to increased azole use for treatment of patients, but also to agricultural use of azoles as fungicides in certain countries.

INTRODUCTION

There have been important advances in the diagnosis and treatment of both the endemic and the opportunistic mycoses, in the delineation of the epidemiology of invasive pulmonary mould infections in immunocompromised patients and in the taxonomy of both yeasts and moulds that cause pulmonary infection. In the last decade, less well-known moulds, such as members of the *Fusarium* and *Scedosporium* genera, are increasingly found to cause invasive pulmonary infection. Newly described *Aspergillus* species that are pathogenic for humans are inherently more drug resistant, and acquired resistance is increasing among the usual species that cause human infection. *Cryptococcus gattii* has emerged in North America as a cause of pulmonary and central nervous system (CNS) infections. Understanding of many of these phenomena had to await the development of molecular methods that could be applied to fungal genomes.

Building on knowledge accrued in the last several decades regarding the cell wall composition of *Aspergillus* and the endemic fungi, *Histoplasma capsulatum* and *Blastomyces dermatitidis*, diagnostic tests for detecting these fungi have become a routine part of the approach to diagnosis. Antigen detection can be accomplished in urine, serum and body fluids, including respiratory samples obtained by bronchoalveolar lavage (BAL). Especially in immunocompromised patients, in whom antibody responses are notoriously poor, antigen detection has led to earlier diagnosis and improved outcomes.

In addition to improved diagnostic techniques, the introduction of new antifungal agents in the last decade has occurred because of studies detailing the synthesis of cell membranes and cell walls in yeasts and moulds. Armed with the knowledge of the action of fluconazole, voriconazole was synthesized specifically to allow more avid binding to the cell membrane of moulds, allowing an increase in the antifungal

spectrum of activity. The new generation azole agents, voriconazole and posaconazole, have allowed safer and more effective treatment of many fungal infections, especially treatment of invasive mould infections in immunocompromised patients. Basic knowledge of cell wall synthesis in *Aspergillus* and *Candida* led to the development of the echinocandins, which target cell wall, rather than cell membrane components. By targeting a structure not shared with mammalian cells, the echinocandins, caspofungin, micafungin and anidulafungin are extremely safe agents for the treatment of invasive fungal infections.

This chapter will review new developments over the last decade in these areas in the context of the major fungal opportunists and endemic mycoses, and will focus on pulmonary aspects of infections with these fungi. The whole panoply of *Aspergillus* pulmonary infections can not be addressed in this review, but rather, the focus will be on invasive disease in immunocompromised hosts.

MAJOR OPPORTUNISTIC MYCOSES

Aspergillosis

Epidemiology

Invasive aspergillosis is by far the most common opportunistic mould causing pulmonary infection. There are over 900 different species of *Aspergillus*, most of which do not cause human infection.[1] Among the species pathogenic for humans, *A. fumigatus* is most commonly isolated, followed by *A. flavus*. There are both experimental and clinical data suggesting that *A. flavus* is more virulent than *A. fumigatus*, but the reasons for this have not been elucidated.[1,2] *A. niger* is usually a respiratory tract commensal and rarely causes invasive infection. Two emerging species that cause invasive pulmonary infection in highly immunosuppressed patients are *A. terreus*, which is inherently amphotericin B resistant, and *A. ustus*, which appears to be inherently resistant to many azoles, as well as amphotericin B.[3]

Aspergillus species are ubiquitous in the environment and only cause invasive disease in hosts that have structural lung disease or defects in immune function that allow germination of spores into tissue-invasive hyphae.[4] Risk factors for invasive pulmonary aspergillosis include haematopoietic cell transplant (HCT) or solid organ transplant, neutropenia, especially when prolonged, corticosteroids and cytomegalovirus infection. Allogeneic HCT are at high risk, especially if they develop chronic graft-versus-host disease, and lung transplant recipients are at great risk because the transplanted organ interfaces with this ubiquitous environmental mould.[1] Increasingly, reports are documenting the emergence of invasive pulmonary aspergillosis in the ICU setting in hosts not classically thought to be immunosuppressed.

Brief overview of invasive pulmonary aspergillosis

Patients usually have symptoms of fever and cough initially; chest pain and haemoptysis follow as the infection progresses. Other symptoms ensue if dissemination to skin, brain or other organs occurs. The clinical presentation varies based on host risk factors. For example, approximately 75% of *Aspergillus* infections in lung transplant recipients are limited to the lungs, whereas disseminated infection is more common in those who have a haematological malignancy.[5,6] Spread to the CNS is a dreaded complication because of the very high mortality rate.

Radiographic techniques have improved over the last several decades and findings highly suggestive of invasive aspergillosis have been established in neutropenic patients; these include nodules surrounded by ground glass, the so-called 'halo sign', caused by haemorrhage, and later, in the course of illness, cavitation of the nodules, causing the so-called 'air crescent sign.'[7] Lung transplant recipients may not have the typical radiological findings noted for patients who have haematological malignancy accompanied by neutropenia. The findings are generally less specific patchy infiltrates and consolidation.[8]

Diagnostic considerations

The diagnosis of invasive pulmonary aspergillosis has been a persistent challenge. Patients are often minimally symptomatic early in the disease when infection might be most amenable to treatment. The clinical presentation and radiographic findings are not specific but are shared with other mould infections. Additionally, the microbiological diagnosis of aspergillosis is limited by the difficulty in obtaining appropriate biopsy material in the patient population most at risk for invasive pulmonary disease.

There have been a number of recent developments to aid clinicians in the diagnosis of pulmonary aspergillosis (Table 1).[9] The most important of these has been the availability of an assay for the detection of *Aspergillus* galactomannan in serum and BAL fluid. Galactomannan is a polysaccharide cell wall component of *Aspergillus* species that is released during the growth cycle. An enzyme immunoassay (EIA) (Platelia, Bio-Rad, Redmond, WA, USA) for this cell wall component in serum has become a standard diagnostic tool for invasive aspergillosis in the last decade.[10,11] The results are reported as an index value, and a positive index is considered to be >0.5 in the United States. The galactomannan assay in serum has proved most helpful when used in the HCT population and when serial testing is performed during the period of highest risk for invasive aspergillosis.[12] In patients who have haematological cancers, including those who have undergone HCT, the serum galactomannan assay has been shown to correlate with outcome.[13] However, in solid organ transplant recipients, the serum assay has been less useful.[14]

The newest diagnostic tool is the detection of *Aspergillus* galactomannan in BAL fluid. Not

Table 1 Methods available for the diagnosis of pulmonary fungal infections

Fungal infection	Diagnostic methods	Comments
Aspergillosis	Culture	Easily grown in lab
	Histopathology	Septate hyphae, but not specific for *Aspergillus*
	CT finding of halo sign	Suspect angioinvasive mould, but not specific
	Galactomannan EIA in serum and BAL	Has become standard test for aspergillosis in serum and likely will become standard in BAL
	RT-PCR in blood and BAL (not standardized)	Likely to play an important role in future
Mucormycosis	Culture	Culture requires special techniques for growth
	Histopathology	Broad non-septate hyphae presumptive evidence for mucormycosis
	CT finding of reverse halo sign	Suggestive only, not specific
	RT-PCR (not standardized)	Perhaps will play a larger role in future
Fusariosis	Culture	Easily grown in lab
	Histopathology	Septate hyphae resemble *Aspergillllus*
	Characteristic skin lesions	Painful lesions with hyphae seen on biopsy
	PCR and *in situ* hybridization (not standardized)	Both perhaps will play a larger role in future
Scedosporiosis	Culture	Easily grown in lab
	Histopathology	Septate hyphae resemble *Aspergillllus*
	PCR and *in situ* hybridization (not standardized)	Both perhaps will play a larger role in future
Cryptococcosis	Culture	Easily grown in lab
	Histopathology	Yeast forms with clear surrounding area are suggestive; mucicarmine stain is specific
	Antigen latex agglutination assay in serum, CSF, body fluids	Highly sensitive and . . . specific; yield lower . . . yield lower with pulmonary infection than with disseminated and CNS infection
Blastomycosis	Culture	Takes 2–4 weeks to grow
	Histopathology	Characteristic yeast forms provide presumptive evidence of infection
	Antigen EIA in urine, serum and perhaps body fluids	Rapid, sensitive, cross-reacts with histoplasmosis
	RT-PCR (not standardized)	Might play a role in future
Histoplasmosis	Culture	Takes 4–6 weeks to grow
	Histopathology	Characteristic yeast forms provide presumptive evidence of infection
	Serology	Useful for acute and chronic pulmonary infection; poor in immunocompromised host
	Antigen EIA in urine, serum BAL, other body fluids	Rapid, sensitive, cross-reacts with blastomycosis
	RT-PCR (not standardized)	Might play a role in future
Coccidioidomycosis	Culture	Easily grown; dangerous for lab; must send to reference lab for identification for safety reasons
	Histopathology	Spherules large and specific; give presumptive diagnosis
	Serology	Very useful for all forms of pulmonary infection
	Antigen EIA in urine, serum	Not clear how sensitive or specific

BAL, bronchoalveolar lavage; CNS, central nervous system; CSF, cerebrospinal fluid; CT, computed tomography; EIA, enzyme immunoassay; RT-PCR, real-time polymerase chain reaction.

surprisingly, it appears to be more sensitive than the serum assay, but the sensitivity depends upon the population studied.[15–18] The highest sensitivity (>90%) has been reported among patients who have haematological diseases;[18] in solid organ transplant recipients, the sensitivity was 82%, compared with 73% for culture and/or microscopy in one series.[16] There are an increased proportion of false positive tests when the assay is performed on BAL fluid, most likely reflecting colonization, especially among lung transplant recipients.[15] Some authors suggest that increasing the index cut-off for positivity to 1.0 instead of 0.5 will improve specificity with only negligible effects on sensitivity.[17,18]

The last decade also has seen a rise in studies using real-time polymerase chain reaction (PCR) on serum,

BAL fluid and tissues as a diagnostic modality. Most studies test for a pan-*Aspergillus* PCR that targets ribosomal DNA common to all *Aspergillus* species; however, primers that are used vary from laboratory to laboratory, preventing standardization. A systematic review and meta-analysis noted the heterogeneity of results among different populations that were studied and the lack of standardization of methods.[19] Overall, the sensitivity and specificity for one positive sample being diagnostic were 88% (75–94%) and 75% (63–84%), respectively A recently approved commercially available PCR assay for aspergillosis (MycAssay Aspergillus, Myconostica, Manchester, UK) was found to have sensitivity and specificity of 70% and 90%, respectively.[20] Not surprisingly, the sensitivity of PCR is increased when BAL fluid is tested, and the specificity also appears to be high. In several recent reports, the sensitivity and specificity for invasive aspergillosis were 94–100% and 88–99%, respectively.[21,22] Although PCR-based tests are increasingly promising, their use continues to be limited by lack of standardization, and they have not yet found a place in routine clinical practice.

Treatment options

In the last decade, the most striking change in the treatment of invasive pulmonary aspergillosis has been the acceptance of voriconazole as the first-line agent for the treatment of invasive pulmonary aspergillosis (Table 2).[23–25] This treatment has improved outcomes and decreased toxicity when compared with amphotericin B therapy. Posaconazole also has proved effective for the treatment of aspergillosis in a salvage trial, but has not been studied or approved for primary therapy.[26] When amphotericin B is indicated, most physicians use a lipid formulation to decrease the risk of nephrotoxicity. The echinocandins have found a role in the treatment of invasive aspergillosis, but primarily, they are used as second-line agents when voriconazole is not tolerated or in combination with voriconazole in an attempt to enhance antifungal activity.[24,25] Although they are exceedingly safe agents, they are fungistatic, and not fungicidal, against *Aspergillus* species and have not been compared with amphotericin B or voriconazole for primary therapy of invasive aspergillosis. An important international trial to evaluate whether combination therapy with an echinocandin and voriconazole is superior to voriconazole alone for HCT patients has been completed; hopefully, the results will settle the long-standing debate about the efficacy of combination antifungal therapy for aspergillosis for the HCT population.

There have been increasing reports of azole resistance in *Aspergillus* species. On the one hand, this is likely related to increasing use of these agents for both prophylaxis and treatment of fungal infections, but on the other, there are associations with exposure to azole-like compounds used in the agricultural industry in some countries.[27,28] Additionally, some species have intrinsic resistance to various azoles.[3] The primary mechanism of resistance in *Aspergillus*

species appears to be amino acid substitutions or promoter alterations of the *cyp51A* gene, which encodes for lanosterol 14-α-sterol demethylase, the target enzyme of the azoles.[29] Resistance to one azole agent often confers resistance to other azoles, and multi-azole resistance been shown to correlate with clinical failure.[30] The situation in the Netherlands is most interesting in that azole resistance is caused by a mutation that is unique to this outbreak and different from those normally seen, and patients appear to be acquiring the resistant strain from the environment. The Netherlands is densely agricultural, and azole-containing fungicides are commonly used, possibly increasing the development of resistance and subsequent spread of these strains to humans.[27]

Mucormycosis

Epidemiology

Mucormycosis is the term used to describe infections caused by fungi belonging to the order Mucorales. The term zygomycosis has fallen out of favour as newer molecular techniques have abolished the class, *Zygomycetes*. Approximately 75% of mucormycosis cases are caused by three genera, *Rhizopus*, *Mucor* and *Rhizomucor*. Mucormycosis has been increasing in the last two decades and is now the second most common cause of pulmonary invasive mould infections in HCT recipients.[31]

The organisms are ubiquitous in the environment and, when aerosolized, may be inhaled where they can invade into pulmonary tissues in susceptible hosts. The Mucorales species have a particular penchant to cause angioinvasion, resulting in tissue necrosis, which likely impedes the host's immune response. Pulmonary mucormycosis occurs in patients who have the most profound degree of immune dysfunction, especially those who have a haematological malignancy complicated by prolonged neutropenia and those who have received an HCT.[32] Rhino-orbital-cerebral mucormycosis also occurs in these patients, but is more common in patients who have poorly controlled diabetes. Other less common predisposing factors for development of mucormycosis include immunomodulating viral infections, such as cytomegalovirus, trauma, iron overload and intravenous drug use.[32]

The common practice of using voriconazole for prophylaxis in markedly immunosuppressed patients may contribute to an increased risk for development of mucormycosis. This matter is still controversial, but it appears that there is possibly an association of mucormycosis with voriconazole use in the most profoundly immunosuppressed HCT recipients.[33,34]

Brief overview of pulmonary mucormycosis

Pulmonary mucormycosis causes a clinical picture similar to that of pulmonary aspergillosis. The

Table 2 Suggested treatment regimens for pulmonary fungal infections

Fungal infection	Preferred treatment	Step-down and second-line treatment
Aspergillosis	Voriconazole, 4 mg/kg bid IV[†] until stable, then step-down therapy	Step-down: voriconazole, 200 mg bid oral, until lesions resolved Second-line: lipid AmB, 5 mg/kg/d IV, until stable, or posaconazole, 400 mg bid oral,[‡] until lesions resolved
Mucormycosis	Lipid AmB, 5 mg/kg/d IV, or higher doses until lesions resolved	Step-down: posaconazole, 400 mg bid oral,[‡] until lesions resolved Second-line: AmB-d, 1 mg/kg/d IV, until lesions resolved (possible addition of echinocandin to AmB formulation)
Fusariosis	Lipid AmB, 5 mg/kg/d IV, or voriconazole, 4 mg/kg bid IV,[†] or combination of lipid AmB, 5 mg/kg/d IV, plus voriconazole, 4 mg/kg bid IV,[†] until stable, then step-down therapy	Obtain susceptibilities because resistance is common Step-down: voriconazole, 200 mg bid oral, until lesions resolved Second-line: posaconazole, 400 mg bid oral,[‡] until lesions resolved
Scedosporiosis	Voriconazole, 4 mg/kg bid IV,[†] until stable, then step-down therapy	Obtain susceptibilities because resistance is common Step-down: voriconazole, 200 mg bid oral, until lesions resolved Second-line: posaconazole, 400 mg bid oral,[‡] until lesions resolved
Cryptococcosis	Severe or with dissemination: lipid AmB, 3–5 mg/kg/d IV + flucytosine, 25 mg/kg qid oral, for 2–4 weeks	Step-down: fluconazole, 400 mg qd oral, for 8–10 weeks, then fluconazole, 200 mg for 6–12 months Second-line: AmB-d, 0.7–1 mg/kg/d IV + flucytosine, 25 mg/kg qid oral, for 2–4 weeks, followed by same step-down therapy as above (For other second-line options and differences in different hosts, see Perfect *et al.*[65])
	Mild to moderate: fluconazole, 400 mg qd oral, for 6–12 months	Itraconazole, 200 mg bid oral,[§] or voriconazole, 200 mg bid oral,[¶] or posaconazole, 400 mg bid oral,[‡] for 6–12 months
Blastomycosis	Severe or immunocompromised: lipid AmB, 3–5 mg/kg/d IV, until stable, then step-down therapy	Add steroids for ARDS Step-down: itraconazole, 200 mg bid oral,[§] 6–12 months Second-line: AmB-d, 0.7–1 mg/kg/d, until stable, then itraconazole, 200 mg bid, oral,[§] for 6–12 months
	Mild to moderate infection: itraconazole, 200 mg bid oral,[§] for 6–12 months	Voriconazole, 200 mg bid oral,[¶] or fluconazole, 800 mg qd oral, for 6–12 months
Histoplasmosis	Severe or immunocompromised: lipid AmB, 3–5 mg/kg/d IV, until stable, then step-down therapy	Add steroids for ARDS Step-down: itraconazole, 200 mg bid oral,[§] 6–12 months Second-line: AmB-d, 0.7-1 mg/kg/d, IV until stable, then itraconazole, 200 mg bid oral,[§] for 6–12 months
	Mild to moderate infection: itraconazole, 200 mg bid oral,[§] for 6–12 months	Voriconazole, 200 mg bid oral,[¶] or fluconazole, 800 mg qd oral, for 6–12 months
Coccidioidomycosis	Severe or immunocompromised: lipid AmB, 3–5 mg/kg/d IV, until stable, then step-down therapy	Step-down: itraconazole, 200 mg bid oral,[§] or fluconazole, 400 mg qd oral, for 12 months Second-line: AmB-d, 0.7-1 mg/kg/d IV, until stable, then itraconazole, 200 mg bid oral,[§] or fluconazole, 400 mg qd oral, for 12 months
	Mild to moderate infection: itraconazole, 200 mg bid oral,[§] or fluconazole, 400 mg qd oral, for 6 months	Voriconazole, 200 mg bid oral,[¶] or posaconazole, 400 mg bid oral,[‡] for 6 months

[†] A loading dose of 6 mg/kg bid IV for the first day should be given.

[‡] Posaconazole can also be dosed as 200 mg qid oral as long as the patient is able to comply with frequent dosing; generally, levels are higher with more frequent dosing.

[§] A loading dose of 200 mg tid oral for the first 3 days should be given.

[¶] A loading dose of 400 mg bid oral for the first day should be given.

Measurement of serum concentrations of itraconazole, voriconazole and posaconazole should be performed when available to ensure adequate absorption, look for drug interactions that might influence serum concentrations and avoid toxicity. Suggested serum levels to aim for are: itraconazole, 1–10 μg/mL; voriconazole, 1–5.5 μg/mL; posaconazole, >1 μg/mL. There is no need to obtain serum concentrations for fluconazole.

AmB, amphotericin B; AmB-d, amphotericin B deoxycholate; bid, two times a day; IV, intravenous; qd, every day; qid, four times a day; tid, three times a day.

infection tends to be rapidly progressive with prominent angioinvasion. Patients complain of dyspnoea, cough, haemoptysis and chest pain, and they usually appear quite ill. Massive haemoptysis can occur. Less often, and mostly in less immunocompromised patients, the disease can be more indolent, presenting with mass-like lesions that can cavitate. Involvement of adjacent structures, including pleura, pericardium and mediastinum is common.

Computed tomography (CT) scans should be obtained at the first hint of pulmonary symptoms in immunocompromised patients who are at risk for mucormycosis. In patients who have haematological malignancies, several diagnostic clues on CT scan recently have been proposed to help differentiate mucormycosis from infection with other angioinvasive moulds, especially aspergillosis. These include the presence of multiple nodules and pleural effusions and the development of a 'reverse halo sign' in patients who have mucormycosis.[7,35] The reverse halo sign appears as ground-glass attenuation in the centre of a nodule with a surrounding zone of consolidation, the obverse of the typical 'halo sign' seen more frequently with other moulds.[7] None of these radiographic findings is specific enough to make a definitive diagnosis of mucormycosis, but should help in the choice of empiric antifungal therapy until a definitive diagnosis can be made.

Mortality rates as high as 70% have been noted in patients who have invasive pulmonary mucormycosis.[32] The high mortality rates likely reflect the poor host response, the difficulty in obtaining an early diagnosis and the limited therapeutic armamentarium. However, recent series show improved outcomes, perhaps related to improvements in diagnosis, allowing earlier treatment and the use of new antifungal agents in the last decade.[36,37]

Diagnostic considerations

The definitive diagnosis of mucormycosis requires the identification of characteristic broad, non-septate hyphae invading tissues, as well as a positive culture yielding a member of the Mucorales order (Table 1). However, using standard procedures for culture that involve homogenization of tissue samples before plating on agar, the recovery rate is only 30–50%. When mucormycosis is a possibility, tissue obtained by biopsy should be processed so that the fragile hyphae are not destroyed; the sample should be very carefully minced and placed directly on agar.[38] In contrast, spores from these organisms are ubiquitous and frequently contaminate culture plates in the laboratory. Thus, biopsy material to ascertain the presence of hyphae in tissues is important to evaluate a culture growing a Mucorales. Unfortunately, patients likely to develop pulmonary mucormycosis are often thrombocytopenic and can not safely undergo lung biopsy. In an immunosuppressed patient whose CT scan shows findings typical for an angioinvasive mould infection, a sputum or BAL culture yielding a Mucorales is adequate to initiate empiric treatment for pulmonary

mucormycosis.[10] This is not the case in a non-immunocompromised host, in whom a positive culture from sputum is more likely to reflect only contamination.

Unfortunately, there are no serological or antigen detection assays available for mucormycosis. The most promise has been shown with PCR assays that can detect Mucorales to the species level in tissues.[39,40] Unfortunately, none of the PCR assays have been standardized, and only a few reference laboratories are able to perform this procedure.

Treatment options

The treatment of mucormycosis is complex given the reduced susceptibility of many species to currently available antifungal agents and the extent of necrosis that often occurs. There are no consensus guidelines for management, but individual approaches have been published.[41] The mainstay of therapy remains amphotericin B (Table 2). New developments in treatment include the almost uniform use of lipid formulations of this agent, rather than the original deoxycholate formulation, for primary therapy and the use of the extended spectrum azole, posaconazole, for step-down therapy. Many clinicians advocate higher doses of amphotericin B for the treatment of mucormycosis than would be used for other indications, and there are experimental data that corroborate this approach.[42] Others argue that this merely enhances the toxicity without clinical benefit. No controlled trials exist to answer this question.

Posaconazole is currently only available as a poorly absorbed oral suspension and thus can not be first-line therapy for invasive mucormycosis. This agent must be given 2–4 times daily with high fat meals or supplements to enhance absorption.[43] This is particularly problematic in neutropenic patients who have mucositis and in HCT recipients who have gastrointestinal graft-versus-host disease. The promise of an intravenous formulation of posaconazole appears to be close to reality, and this could change the approach to therapy if initial studies show efficacy.

Voriconazole has no activity against the Mucorales. The common use of voriconazole for empiric therapy in immunocompromised patients who have suspected invasive mould infections has the potential to increase mortality in patients who have mucormycosis because it delays the use of an effective antifungal agent.[37]

There have been several adjunctive measures in the treatment of mucormycosis brought forth in the last decade. These include the addition of echinocandins, the iron chelator, deferasirox and several different immunomodulatory agents to amphotericin B therapy.[44] Echinocandins do not have activity against the Mucorales *in vitro*, but there are some theoretical reasons and experimental animal data noting that these agents might be helpful when used in combination with amphotericin B. In a small retrospective series of patients with rhino-orbital-cerebral

mucormycosis, dual therapy with an echinocandin and lipid amphotericin appeared to have some benefit.[44] This approach has not been studied in patients with pulmonary mucormycosis and is not generally recommended.

Some Mucorales are highly dependent on iron for growth. Fungicidal effects against *Rhizopus* species have been noted *in vitro* and in animal models by the iron chelator, deferasirox. This agent has been given, along with amphotericin B, to a few patients with generally favourable results.[44] A clinical trial to study the safety and efficacy of this treatment strategy has been completed, but early evidence appears to show no benefit. Immunotherapy with interferon-gamma and/or granulocyte macrophage colony-stimulating factor has been a promising possibility for the last decade but has not gained widespread acceptance at this time.

Other hyaline moulds (*Fusarium* and *Scedosporium*)

Epidemiology

There are at least 100 *Fusarium* species, most of which are plant pathogens; only a few species cause human disease. *F. solani*, *F. oxysporum* and *F. moniliforme* account for more than 90% of human infections.[45] Infections in non-immunocompromised patients are usually localized and related to direct inoculation. In severely immunocompromised patients, pulmonary infection is a common manifestation and occurs either in the setting of haematogenous spread during disseminated disease or from inhalation of conidia into the lungs.

Scedosporium species are ubiquitous moulds that are found in soil and water. The nomenclature has been confusing and has been revised recently using molecular methods. The clinically relevant species are *S. prolificans* and the *S. boydii* (or, if in the sexual stage, *Pseudallescheria boydii*) complex, which includes *S. boydii*, *S. aurantiacum* and *S. apiospermum*.[46] *S. prolificans* differs from other *Scedosporium* species in that it produces melanin, a common fungal virulence factor, is able to sporulate *in vivo* similar to *Fusarium* species and is resistant to almost all antifungal agents.[47]

In the last decade, *Scedosporium* species have been increasingly reported as a cause of invasive pulmonary infections in solid organ transplant recipients, HCT recipients and patients with haematological malignancies.[48,49] Pulmonary infection usually occurs from inhalation of conidia into the alveoli but can occur from haematogenous spread in patients with disseminated disease.

The pathogenesis of infection with either *Fusarium* species or *Scedosporium* species is likely similar to that seen with aspergillosis, but infection in humans has not been studied as intensively as aspergillosis. Many *Fusarium* species produce a variety of toxins, but it is unclear what role, if any, they play in invasive pulmonary infection.[50]

Brief overview of pulmonary disease

The clinical presentation of pulmonary infection with *Fusarium* and *Scedosporium* is similar to that noted with *Aspergillus*. Patients present with fever, cough, pleuritic chest pain and dyspnoea. CT scans usually show nodules, often surrounded by ground-glass opacities (halo sign) signifying angioinvasion with resultant haemorrhage. Disseminated infection is the rule with *Fusarium* infections, and non-pulmonary manifestations can provide valuable clinical clues to the diagnosis of this infection. Most often this is the appearance of painful skin lesions that are manifested as target lesions, necrotic ecthyma-like lesions, ulcerations or pustules.[51] Additionally, in neutropenic patients, *Fusarium* species cause paronychia and local cellulitis, which is often a harbinger of disseminated fusariosis.

Diagnostic considerations

There are currently no serological or antigen detection tests available to aid in the diagnosis of pulmonary fusariosis or scedosporiosis. The definitive diagnosis is established by growing the organism in the laboratory (Table 1). The clinician is aided in this endeavour by the fact that *Fusarium* species and *S. prolificans* (but rarely organisms of the *S. boydii* complex) are some of the very few moulds that are capable of growing in blood culture bottles. As many as 40% of patients with disseminated fusariosis have been noted to have positive blood cultures.[50] For both genera, histopathological examination of infected tissue demonstrates acutely branching septate hyphae that are often undistinguishable from those of *Aspergillus* species. This establishes that a patient has disseminated infection with a septate mould, but growth in culture is extremely important to establish which specific organism is causing infection and allow appropriate antifungal therapy. PCR and *in situ* hybridization have been reported to be helpful in the diagnosis of both scedosporiosis and fusariosis, but none of these tests are standardized or approved.[52,53]

Treatment options

Treatment of pulmonary fusariosis remains challenging because many species are highly resistant to antifungal agents. If culture material is available, susceptibility testing is critical for determining optimal antifungal therapy. Several major improvements have been seen in the last decade (Table 2). For some patients, voriconazole and posaconazole have been effective, both as primary treatment and as salvage therapy when other agents have failed.[54,55] Lipid formulations of amphotericin B are still recommended, and some physicians use a combination of a lipid formulation of amphotericin B with voriconazole.[56] There are numerous reports of clinical failure with all of these drugs, however, and ultimately, patients who remain neutropenic are unlikely to respond to any antifungal agent.[49]

Scedosporiosis is difficult to treat because of innate resistance of many isolates to most antifungal agents; importantly, this group of organisms is resistant to amphotericin B (Table 2). *S. prolificans* is clearly more resistant than organisms in the *S. boydii* complex.[47] Voriconazole appears to be the most effective agent in the treatment of *S. apiospermum* infections and has been successful in some *S. prolificans* infections.[57] Posaconazole also has been used in a few patients and appeared to be effective.[43] Treatment of invasive infection with *S. prolificans* remains dismal.[58]

Cryptococcosis

Epidemiology

New developments in the epidemiology of cryptococcosis in the last decade are the acceptance of *C. gattii* as a separate species and the emergence of this organism in the Pacific Northwest and in scattered places throughout the United States. *C. neoformans* remains the most prevalent human pathogen in this genus and is found worldwide in the environment. *C. gattii* formerly was reported mostly from Australia and other subtropical and tropical areas and was linked to eucalyptus trees in these areas. The outbreak of *C. gattii* in North America began on and around Vancouver Island, British Columbia, about 12 years ago, is not related to eucalypts and has subsequently spread southward. Genotyping has shown that one strain predominates in the Pacific Northwest, while other genotypes are more common elsewhere.[59]

Brief overview of pulmonary cryptococcosis

Pulmonary cryptococcosis occurs in 'normal' hosts as well as those who are immunocompromised. Not surprisingly, the disease is generally more severe in those who have defects in immunity and often accompanied by disseminated infection, including CNS disease. In immunocompetent hosts, cryptococcosis is more likely to be limited to the lung and occurs more commonly in patients with chronic lung disease.[60] Solid organ transplant recipients generally develop cryptococcosis late after transplantation, and about a third will have isolated pulmonary infection.[61] The species, and perhaps even the strain, of *Cryptococcus* also has an impact on pulmonary infection; in the recent *C. gattii* outbreak, pulmonary infection was noted more frequently than previously reported with this species.[59]

Immunocompetent subjects with pulmonary cryptococcosis usually are asymptomatic or have mild symptoms that include fever, malaise, cough with sputum production and perhaps dyspnoea. Immunocompromised patients are much more likely to present with fulminant infection with fever, dry cough and dyspnoea that can progress to acute respiratory distress syndrome (ARDS).

Imaging studies show solitary pulmonary nodules, often pleural-based, diffuse nodules, consolidation or diffuse bilateral infiltrates.[62,63] Lymphadenopathy and cavitation occur but are uncommon.

Diagnostic considerations

The diagnosis of pulmonary cryptococcal infection is confirmed if the organism is grown in culture from sputum or BAL fluid in a patient who has clinical symptoms and radiographic findings compatible with cryptococcosis (Table 1). In a patient who has no radiographic findings, isolating *C. neoformans* from sputum may reflect colonization. *Cryptococcus* species grow readily on standard agar media and can be easily identified by clinical laboratories. In tissues, a clear zone, representing the capsule around the yeast, is sometimes seen. A stain specific for *Cryptococcus*, the mucicarmine stain, should always be performed to establish the identity of yeast-like structures seen in tissues.

The cryptococcal antigen test is highly sensitive and specific and is routinely used to help establish the diagnosis of cryptococcal meningitis and disseminated cryptococcosis. However, the serum cryptococcal antigen test is often negative in patients who have isolated pulmonary infection. In fact, a positive serum cryptococcal antigen test in a solid organ transplant recipient who has pulmonary cryptococcosis has been shown to reflect extrapulmonary or more severe pulmonary disease.[61]

Because of the frequency of disseminated infection in immunocompromised patients, a systematic evaluation, including cultures from blood and cerebrospinal fluid and measurement of serum and cerebrospinal fluid cryptococcal antigen, should be performed in all immunosuppressed patients who present with seemingly isolated pulmonary cryptococcosis.[64]

Treatment options

Little has changed in the treatment of pulmonary cryptococcosis due to either *C. neoformans* or *C. gattii* in the last decade (Table 2).[65] Therapy is dependent upon whether the patient has isolated pulmonary infection or has dissemination to other organs, especially the CNS, and is also dependent on whether the patient is immunosuppressed. In any patient who is immunosuppressed, a lumbar puncture should be performed to look for meningitis. If the patient is immunocompetent, has minimal pulmonary disease with no symptoms suggesting CNS infection and has a negative test for cryptococcal antigen in serum, many physicians feel comfortable with not performing a spinal tap because it is highly unlikely that the patient has CNS involvement.[63]

For mild to moderate isolated pulmonary infection, oral fluconazole for 6–12 months is recommended.[65] For severe pulmonary involvement, or when concomitant meningitis or dissemination to other organs is present, therapy is the same as for CNS disease: initial treatment with amphotericin B combined with flucytosine for 2–4 weeks, followed by consolidation

therapy with fluconazole for an additional 8–10 weeks and then maintenance fluconazole therapy for 6–12 months. Although amphotericin B deoxycholate is recommended in the Infectious Diseases Society of America (IDSA) Guidelines,[65] many physicians prefer to use a lipid formulation of amphotericin B to decrease the risk of nephrotoxicity.

MAJOR ENDEMIC MYCOSES

Blastomycosis

Epidemiology

B. dermatitidis, the causative agent of blastomycosis, is a dimorphic fungus that exists as a mould in the environment and as a yeast in tissues at 37°C. Most cases occur in persons living in states that border the Mississippi River basin, the Great Lakes and the St Lawrence Seaway, and in the Canadian provinces of Ontario and Manitoba.[66] The environmental niche for *B. dermatitidis* appears to be soil and decaying wood, especially along waterways. Most cases are sporadic, but outbreaks have been reported.

Brief overview of pulmonary blastomycosis

Infection begins with inhalation of conidia into the alveoli, at which point the organism converts to the yeast form. It is likely that haematogenous dissemination occurs in most patients, but clinical manifestations of this event, as well as the initial pulmonary infection, are uncommon. Both neutrophils and cell-mediated immunity are important in the response to *B. dermatitidis*, and the histopathological picture is a mixed pyogranulomatous process.[66]

Most patients with acute pulmonary infection with *B. dermatitidis* remain asymptomatic or have a mild illness with fever, dry cough, dyspnoea and mild chest pain. A localized pulmonary infiltrate that is often nodular is noted on chest radiograph.[67] A small proportion of patients with acute blastomycosis develop overwhelming infection with ARDS.[68] This is seen most often in immunocompromised patients. However, healthy adults, presumably who had exposure to a large number of conidia, can also develop ARDS. The mortality rate is extremely high in this form of pulmonary blastomycosis.

Chronic forms of pulmonary blastomycosis can present as mass-like lesions that resemble lung cancer or as upper lobe cavitary lesions that resemble tuberculosis or histoplasmosis. Fever, night sweats, weight loss, fatigue, dyspnoea and cough with purulent sputum and haemoptysis, are usually present for weeks. Hilar and mediastinal lymphadenopathy are seen less commonly than in histoplasmosis.

Cutaneous lesions are the most common manifestation of disseminated extra-pulmonary blastomycosis; prostate and osteoarticular involvement are less common. The cutaneous lesions are frequently multiple, usually well circumscribed and can be verrucous or ulcerated. When they appear during the course of pulmonary infection, they provide a diagnostic clue to the aetiology of the pneumonia; however, often the pneumonia has cleared before the skin lesions are manifested.

Diagnostic considerations

Growth of *B. dermatitidis* from a tissue biopsy or body fluid sample sent to the laboratory remains the definitive diagnostic test for blastomycosis (Table 1). The organism takes several weeks to grow. Once growth occurs, identification is made quickly with a highly specific DNA probe. However, for many patients, the diagnosis can not be delayed for weeks and reliable rapid tests are essential to be able to initiate appropriate treatment as soon as possible.

Available rapid tests include histopathological examination of tissues, cytological examination of body fluids, such as sputum or BAL and antigen detection.[69] The organisms are larger than most yeasts (8–10 μm), thick-walled and have a single broad-based bud. For an experienced pathologist, this specific morphology distinguishes *B. dermatitidis* from other yeasts and is adequate to initiate therapy. However, confirmation by growth of the organism still should be pursued.

The newest development in the diagnosis of blastomycosis is an EIA performed on urine or serum that detects a cell wall galactomannan antigen found in *B. dermatitidis*.[70,71] The cell wall galactomannans for *H. capsulatum* and *B. dermatitidis* are very similar, and the cross-reactivity between the two assays is close to 100%. The *Blastomyces* assay also cross-reacts with antigens from *Paracoccidioides brasiliensis* and *Penicillium marneffei*; however, these infections differ clinically from blastomycosis and occur in different geographic regions. Recent modifications in the methods have improved the sensitivity of the test on serum.

Antigen can be found in both disseminated and pulmonary forms of blastomycosis.[71,72] In one series of 27 patients who had documented blastomycosis, 21 of 26 (81%) had antigen detected in urine, and 9 of 11 (82%) had antigen detected in serum.[71] The test is probably most useful in patients who have more severe disease with a higher burden of organisms. It is presumed that the response to therapy can be followed using the *Blastomyces* EIA, but there is minimal experience in using this assay for follow-up testing.

A real-time PCR assay for *B. dermatitidis* has been reported recently. Specificity was found to be 99% and sensitivity 86% when compared with culture results. Most clinical specimens were from the respiratory tract, but specifics were not reported and the number of patients with culture-positive blastomycosis was only 14.[73]

Treatment options

All patients with symptomatic blastomycosis should be treated with an antifungal agent. Patients who have only a single cutaneous lesion or other focal

manifestation should be treated for disseminated blastomycosis. The updated IDSA guidelines for the management of blastomycosis reflect several new aspects of treating this disease (Table 2).[74] The major change is that treatment solely with a prolonged course of amphotericin B deoxycholate is no longer recommended as first-line therapy for any form of blastomycosis. Patients who have severe infection and those who are immunosuppressed are treated initially with amphotericin B until stable and then changed to an oral azole, usually within several weeks. The guidelines suggest that either amphotericin B deoxycholate or a lipid formulation can be used, but in fact, most physicians now use a lipid formulation because of the lessened toxicity of these formulations.

The second change is the increased use of azole agents as sole therapy for mild to moderate pulmonary blastomycosis. The primary agent recommended is itraconazole. After the guidelines were published, an increasing number of reports on the use of voriconazole for blastomycosis appeared.[67] This agent is considered to be second-line but has proven effective for treating CNS infections, as well as infection in immunosuppressed patients. Fluconazole is another second-line agent;[74] there is little experience with posaconazole for blastomycosis.

Finally, it is important to note that some clinicians use intravenous methylprednisolone as adjunctive therapy to amphotericin B in patients who have ARDS secondary to blastomycosis. Individual case reports show success with this approach.[75] This practice is commented upon in both the IDSA and the American Thoracic Society Guidelines, but it is not endorsed because of insufficient evidence.[25,74]

Histoplasmosis

Epidemiology

The causative agent of histoplasmosis, *H. capsulatum*, is a temperature-dependent dimorphic fungus, existing as a mould in the environment and a yeast in tissues at 37°C.[76] *H. capsulatum* is found primarily in the Ohio and Mississippi River valleys and in Central America, but other localized foci also exist in Europe, Africa and Asia. The environmental niche for *H. capsulatum* is soil that is enriched by the nitrogen contained in bird and bat guano. Abandoned buildings and areas under trees that serve as bird and bat roosts and caves are especially likely to contain high concentrations. In the endemic areas, exposure to *H. capsulatum* is common, and most infections are sporadic. Outbreaks have been traced back to spelunking, demolition of buildings and activities that disrupt contaminated soil.

Brief overview of pulmonary histoplasmosis

Infection begins when the microcondia of *H. capsulatum* are inhaled into the alveoli. Local lymphatic spread and haematogenous dissemination occur in most persons but are asymptomatic in most. The major host defence against *H. capsulatum* is cell-mediated immunity. The extent of disease is determined both by the number of conidia inhaled and the immune response of the host. A small inoculum can cause severe infection in markedly immunosuppressed hosts. Conversely, healthy individuals, who most commonly have asymptomatic pulmonary infection, can develop life-threatening pneumonia if they inhale a large quantity of conidia.

Pulmonary infection with *H. capsulatum* can be manifested as an acute self-limited pneumonia characterized by fever, dry cough, mild chest discomfort and fatigue.[76,77] The chest radiograph reveals a patchy infiltrate, and the CT scan frequently reveals small nodules; hilar lymphadenopathy may be present and is helpful in differentiating histoplamosis from bacterial pneumonias. In some patients, symptoms are protracted, and both symptoms and radiographic findings persist for months. A small minority of patients develops severe pneumonia that can progress to ARDS. This is seen most often in immunosuppressed patients and in those who had an overwhelming exposure to the organism.

Chronic cavitary pulmonary histoplasmosis is a disease of older adults who have underlying emphysema. Symptoms are present for months and include fever, fatigue, anorexia, weight loss, cough with purulent sputum and haemoptysis. On chest radiographs and CT scans, unilateral or bilateral upper lobe infiltrates and thick-walled cavities are noted. Extensive fibrosis and scarring are commonly seen.[76]

Uncommonly, patients may have persistent mediastinal and/or hilar lymphadenopathy following acute pulmonary histoplasmosis. Although most patients with this complication are asymptomatic, the nodes can impinge on thoracic structures causing dysphagia, chest pain, dyspnoea and cough. Mediastinal fibrosis is a rare complication of pulmonary histoplasmosis in which the host responds to infection with excessive fibrosis that can culminate in constriction of the great vessels and bronchi.[77] Symptoms include dyspnoea, cough, wheezing and haemoptysis. CT scan shows the extent of the fibrosis, and angiographic studies are essential to evaluate involvement of the major thoracic blood vessels.

Disseminated disease occurs in a minority of patients with histoplasmosis; most often, this occurs in immunosuppressed individuals. Pulmonary manifestations of disseminated histoplasmosis vary from subtle diffuse nodules noted in the more chronic form of disseminated infection to life-threatening pneumonia with ARDS noted in immunosuppressed patients with acute disseminated infection.

Diagnostic considerations

Histoplasmosis is definitively diagnosed by growth of the organism (Table 1). Sputum, BAL fluid, lung tissue or mediastinal lymph nodes can be cultured. *H. capsulatum* may take as long as 4–6 weeks to grow at room temperature in the mould form. Tentative identification is made when the characteristic tuberculate macroconidia appear, and definitive identification is

made with the use of a commercially available highly specific DNA probe for *H. capsulatum*.

Identification of the typical small (2–4 μm) oval budding yeasts in tissue or fluid samples allows an early diagnosis while awaiting culture results. Methenamine silver or periodic acid Schiff stains are needed to visualize the organisms. For patients who have disseminated infection in addition to pulmonary infection, bone marrow, lymph nodes, mucous membrane lesions or skin lesions often reveal the organisms and can obviate the need for bronchoscopy.

Serology plays an important role in the diagnosis of chronic cavitary pulmonary and acute pulmonary histoplasmosis. Both complement fixation and immunodiffusion tests should be ordered.[76] For patients with acute pneumonia, the initial studies are often negative, but will show a fourfold rise over the subsequent few weeks. Serology is not as useful in immunosuppressed patients who can not mount an antibody response.

The newest development to aid clinicians in the diagnosis of histoplasmosis is the continuing improvement in the sensitivity of the *Histoplasma* EIA that detects a galactomannan component of the cell wall of *H. capsulatum*. The original assay in urine was shown to be positive in >90% of patients who had AIDS and disseminated histoplasmosis, but did not perform as well in non-AIDS patients and in those with pulmonary infection. With modifications that have increased the sensitivity of both the serum and the urine assays, antigen detection now appears to be increasingly useful for the diagnosis of disseminated infection in non-AIDS patients and for the diagnosis of several pulmonary syndromes.[78–81] In acute pulmonary histoplasmosis, a sensitivity of approximately 65% was noted for both serum and urine assays and was more likely to be positive in those who had more severe infection.[79] Patients who have a subacute course of pulmonary histoplasmosis are likely to have a smaller burden of organisms and are less likely to have a positive antigen assay.[81] BAL fluid can also be tested for *Histoplasma* antigen, and this test appears to be more sensitive than cytology or culture.[80] Cross-reactivity of the *Histoplasma* assay is almost 100% with the assay for *B. dermatitidis* because they share similar galactomannans in their cell walls. The assay also cross-reacts with antigens from *P. brasiliensis* and *P. marneffei*.

A real-time PCR assay for *H. capsulatum* has been reported recently. Specificity was found to be 100% and sensitivity 73% when compared with culture results. Most clinical specimens were from the respiratory tract, but the test performed poorly with BAL fluids for unclear reasons. The number of patients with culture-positive histoplasmosis was only 15, so these results must be viewed as preliminary.[73]

Treatment options

A major change in the last decade has been a shift away from the use of amphotericin B deoxycholate towards the increasing use of azoles for many forms of histoplasmosis (Table 2). However, amphotericin B is still recommended for patients with severe pulmonary histoplasmosis and for immunosuppressed patients. The newest IDSA recommendations are to use liposomal amphotericin B preferentially over the deoxycholate formulation, based on a controlled trial that showed the superiority of this agent in AIDS patients with severe disseminated histoplasmosis.[82]

Azoles are recommended as first-line treatment for patients who have mild to moderate pulmonary histoplasmosis and as step-down therapy after a patient who has severe disease has responded to initial treatment with amphotericin B. Itraconazole is the drug of choice and also is recommended for patients who have chronic cavitary pulmonary histoplasmosis; amphotericin B now is rarely used for this chronic disease.

Another change is the increasing use of voriconazole and posaconazole for histoplasmosis. Many times, these agents are used when a patient either can not tolerate itraconazole or the appropriate serum concentrations can not be achieved. There are no controlled treatment trials, but clinical experience has verified that these newer azoles are effective for histoplasmosis.[83] They remain second-line agents, as does fluconazole.[82]

Reflecting increasing use of corticosteroids for ARDS in the last decade, the current IDSA Guidelines and the American Thoracic Society Guidelines more strongly recommend the use of intravenous methylprednisolone as adjunctive therapy for severe acute pulmonary histoplasmosis.[25,82] Another adjunctive, often life-saving, measure is the increasing practice of placing intravascular stents in order to open major vessels impacted by mediastinal fibrosis.[84] This requires an interventionalist who is experienced in the management of this rare disease.

Coccidioidomycosis

Epidemiology

There are now two distinct species, *Coccidioides immitis* found in southern California and *C. posadasii* found in other areas of the desert southwest and a few areas in Central and South America.[85] The disease caused by these two species is indistinguishable. *Coccidioides* species are dimorphic, existing as moulds in the environment and as spherules *in vivo*. Arthroconidia that develop when the organism is in the mould form are easily dispersed and inhaled into the alveoli. In the lungs, the organism transforms into large thick-walled spherules, which contain hundreds of endospores. When the spherule matures, it ruptures and releases the endospores, which propagate the infection.

The primary host defence against *Coccidioides* species appears to be cell-mediated immunity although neutrophils also are present in most lesions.[85] Haematogenous dissemination presumably occurs in many patients. Dark-skinned races,

especially African Americans, are at higher risk for dissemination and severe infection than other persons.

Brief overview of pulmonary coccidioidomycosis

Most persons infected with *Coccidioides* species have no symptoms or symptoms suggesting mild community-acquired pneumonia.[86] Fever, fatigue, dry cough, anterior chest pain and dyspnoea are common symptoms. Chest radiographs usually show nodular or patchy infiltrates, and hilar lymphadenopathy may be present. Diffuse infiltrates with hypoxemia are uncommon and are usually seen in immunosupppressed patients. Complications following acute pneumonia occur in 5–10% of patients and include solitary thin-walled cavities, chronic progressive cavitary pneumonia and fibrosis.[85] Fever, weight loss, dyspnoea, purulent sputum and haemoptysis are noted in patients who have chronic infection.

Disseminated infection occurs in less than 1% of patients with symptomatic coccidioidomycosis and may be present concomitantly with pulmonary involvement. The sites most often involved are the skin, subcutaneous tissues, osteoarticular structures and meninges. Meningitis can be an isolated finding or one component of widespread dissemination.

Diagnostic considerations

Coccidioidomycosis is definitively diagnosed when the organism is grown in culture (Table 1). *Coccidioides* species grow as a mould within a few days on most standard media. It is important to warn laboratory personnel if coccidioidomycosis is a possibility as the mould form is highly infectious. The large spherules are readily identified in tissues and with the aid of potassium hydroxide or calcofluor white, can be identified in sputum or BAL fluid. This is extremely helpful in establishing an early diagnosis before culture evidence is available.

Serology is very useful in the diagnosis of coccidioidomycosis, especially when a reference laboratory in the endemic area that is experienced in testing for coccidioidomycosis performs the tests.[87] An EIA is available for screening, but the complement fixation and immunodiffusion tests are more specific and sensitive.[78,87] Antibody titers are useful for both acute and chronic pulmonary infections and for disseminated infection. A positive antibody test for *Coccidioides* in cerebrospinal fluid is diagnostic of coccidioidal meningitis; many times this is the only test that is positive in patients with isolated chronic meningitis.

In regard to new diagnostic tests, there is now a commercially available EIA antigen detection test that can be performed on urine or serum; experience with this test is limited at this point. This assay was reported to have a sensitivity of 71% in immunosuppressed patients who had moderately severe to severe coccidioidomycosis.[88] Presumably further refinements will be forthcoming to increase the sensitivity of this assay.

Treatment options

The treatment of coccidioidomycosis remains amphotericin B in patients with severe pulmonary or disseminated infection and oral azole therapy for those with less severe infection (Table 2).[89] Although the IDSA Guidelines published in 2005 recommend amphotericin B deoxycholate, most physicians now use a lipid formulation of amphotericin B to decrease the risk of toxicity. After a clinical response is seen, therapy is changed to an azole. Either fluconazole or itraconazole can be used for step-down therapy, for mild to moderate disease and for complications of pulmonary coccidioidomycosis that require long-term therapy. Treatment should continue for at least 1 year, except in those patients who have mild disease that responds promptly to therapy. Persistent cavitary lesions that remain after adequate azole therapy should be evaluated for possible surgical removal.

In the last decade, there has been increasing use of voriconazole and posaconazole for patients in whom the first-line azole agents have failed or for patients who have become intolerant of these agents. These newer azole drugs probably have been used more in coccidioidomycosis than other endemic infections because of the inherent difficulty in obtaining a lasting remission in this disease and the need to treat with azoles for a prolonged period of time. In a review of 37 patients treated with either voriconazole or posaconazole at one centre and 33 other patients previously reported from other medical centres, a response rate in patients for whom either agent was used, primarily for salvage therapy, was approximately 75% for those with pulmonary coccidioidomycosis.[90] Both voriconazole and posaconazole have also been used successfully for treating coccidioidal meningitis, the most difficult-to-treat form of coccidioidomycosis.

REFERENCES

1 Kauffman CA, Nicolasora NP. Epidemiology of invasive pulmonary aspergillosis. In: Pasqualotto AC (ed.) *Aspergillosis: From Diagnosis to Prevention*. Springer, New York, 2009; 329–44.
2 Pasqualotto AC. Differences in pathogenicity and clinical syndromes due to *Aspergillus fumigatus* and *Aspergillus flavus*. *Med. Mycol.* 2009; **47**: S261–70.
3 van der Linden JWM, Warris A, Verweij PE. *Aspergillus* species intrinsically resistant to antifungal agents. *Med. Mycol.* 2011; **49**: S82–9.
4 Cramer RA, Rivera A, Hohl T. Immune responses against *Aspergillus fumigatus*: what have we learned? *Curr. Opin. Infect. Dis.* 2011; **24**: 315–22.
5 Singh N, Husain S. AST Infectious Diseases Community of Practice. Invasive aspergillosis in solid organ transplant recipients. *Am. J. Transplant.* 2009; **9**(Suppl. 4): S180–91.
6 Ben-Ami R, Lewis R, Kontoyiannis DP. Invasive mould infections in the setting of hematopoietic cell transplantation: current trends and new challenges. *Curr. Opin. Infect. Dis.* 2009; **22**: 376–84.
7 Georgiadou SP, Sipsas NV, Marom EM *et al*. The diagnostic value of halo and reversed halo signs for invasive mold infections in compromised hosts. *Clin. Infect. Dis.* 2011; **52**: 1144–55.

8 Park SY, Kim SH, Choi SH *et al.* Clinical and radiological features of invasive pulmonary aspergillosis in transplant recipients and neutropenic patients. *Transpl. Infect. Dis.* 2010; **12**: 309–15.

9 Segal BH. Aspergillosis. *N. Engl. J. Med.* 2009; **360**: 1870–84.

10 De Pauw B, Walsh TJ, Donnelly JP *et al.* Revised definitions of invasive fungal disease from the 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 (EORTC/MSG) Consensus Group. *Clin. Infect. Dis.* 2008; **46**: 1813–21.

11 Maertens J, Theunissen K, Lodewyck T *et al.* Advances in the serological diagnosis of invasive *Aspergillus* infections in patients with haematological disorders. *Mycoses* 2007; **50** (Suppl. 1): 2–17.

12 Pfeiffer CD, Fine JP, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. *Clin. Infect. Dis.* 2006; **42**: 1417–27.

13 Miceli MH, Grazziutti ML, Woods G *et al.* Strong correlation between serum *Aspergillus* galactomannan index and outcome of aspergillosis in patients with hematological cancer: clinical and research implications. *Clin. Infect. Dis.* 2008; **46**: 1412–22.

14 Husain S, Kwak EJ, Obman A *et al.* Prospective assessment of Platelia *Aspergillus* galactomannan antigen for the diagnosis of invasive aspergillosis in lung transplant recipients. *Am. J. Transplant.* 2004; **4**: 796–802.

15 Clancy CJ, Jaber RA, Leather HL *et al.* Bronchoalveolar lavage galactomannan in diagnosis of invasive pulmonary aspergillosis among solid-organ transplant recipients. *J. Clin. Microbiol.* 2007; **45**: 1759–65.

16 Husain S, Clancy CJ, Nguyen MH *et al.* Performance characteristics of the platelia *Aspergillus* enzyme immunoassay for detection of *Aspergillus* galactomannan antigen in bronchoalveolar lavage fluid. *Clin. Vaccine Immunol.* 2008; **15**: 1760–3.

17 Guo YL, Chen YQ, Wang K *et al.* Accuracy of bronchoalveolar lavage galactomannan in diagnosing invasive aspergillosis: a bivariate meta-analysis and systematic review. *Chest* 2010; **138**: 817–24.

18 Maertens J, Maertens V, Theunissen K *et al.* Bronchoalveolar lavage fluid galactomannan for the diagnosis of invasive pulmonary aspergillosis in patients with hematologic diseases. *Clin. Infect. Dis.* 2009; **49**: 1688–93.

19 Mengoli C, Cruciani M, Barnes RA *et al.* Use of PCR for diagnosis of invasive aspergillosis: systematic review and meta-analysis. *Lancet Infect. Dis.* 2009; **9**: 89–96.

20 White LP, Perry MD, Moody A *et al.* Evaluation of analytical and preliminary clinical performance of Myconostica MycAssay Aspergillus when testing serum specimens for diagnosis of invasive aspergillosis. *J. Clin. Microbiol.* 2011; **49**: 2169–74.

21 Luong M-L, Clancy CJ, Vadnerkar A *et al.* Comparison of an *Aspergillus* real-time polymerase chain reaction assay with galactomannan testing of bronchoalveolar lavage fluid for the diagnosis of invasive pulmonary aspergillosis in lung transplant recipients. *Clin. Infect. Dis.* 2011; **52**: 1218–26.

22 Torelli R, Sanguinetti M, Moody A *et al.* Diagnosis of invasive aspergillosis by a commercial real-time PCR assay for *Aspergillus* DNA in bronchoalveolar lavage fluid samples from high-risk patients compared to a galactomannan assay. *J. Clin. Microbiol.* 2011; **49**: 4273–78.

23 Herbrecht R, Denning DW, Patterson TF *et al.* Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. *N. Engl. J. Med.* 2002; **347**: 408–15.

24 Walsh TJ, Anaissie EJ, Denning DW *et al.* Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. *Clin. Infect. Dis.* 2008; **46**: 327–60.

25 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.

26 Walsh TJ, Raad I, Patterson TF *et al.* Treatment of invasive aspergillosis with posaconazole in patients who are refractory to or intolerant of conventional therapy: an externally controlled trial. *Clin. Infect. Dis.* 2007; **44**: 2–12.

27 Verweij PE, Snelders E, Kema GHJ *et al.* Azole resistance in *Aspergillus fumigatus*: a side effect of environmental fungicide use. *Lancet Infect. Dis.* 2009; **9**: 789–95.

28 Howard SJ, Arendrup MC. Acquired antifungal drug resistance in *Aspergillus fumigatus*: epidemiology and detection. *Med. Mycol.* 2011; **49**: S90–5.

29 Bowyer P, Moore CB, Rautemaa R *et al.* Azole antifungal resistance today: focus on aspergillosis. *Curr. Infect. Dis. Rep.* 2011; **13**: 485–91.

30 van der Linden JWM, Snelders E, Kampinga GA *et al.* Clinical implications of azole resistance in *Aspergillus fumigatus*, the Netherlands, 2007-2009. *Emerg. Infect. Dis.* 2011; **17**: 1846–54.

31 Kontoyiannis DP, Marr KA, Park BJ *et al.* Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001–2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) database. *Clin. Infect. Dis.* 2010; **50**: 1091–100.

32 Roden MM, Zaoutis TE, Buchanan WL *et al.* Epidemiology and outcome of zygomycosis: a review of 929 reported cases. *Clin. Infect. Dis.* 2005; **41**: 634–53.

33 Kontoyiannis DP, Lionakis MS, Lewis RE *et al.* Zygomycosis in a tertiary-care cancer center in the era of *Aspergillus*-active antifungal therapy: a case-control observational study of 27 recent cases. *J. Infect. Dis.* 2005; **191**: 1350–60.

34 Trifilio S, Singhal S, Williams S *et al.* Breakthrough fungal infections after allogeneic hematopoietic stem cell transplantation in patients on prophylactic voriconazole. *Bone Marrow Transplant.* 2007; **40**: 451–6.

35 Chamilos G, Marom EM, Lewis RE *et al.* Predictors of pulmonary zygomycosis versus invasive aspergillosis in patients with cancer. *Clin. Infect. Dis.* 2005; **41**: 60–6.

36 Hammond SP, Baden LR, Marty FM. Mortality in hematologic malignancy and hematopoietic stem cell transplant patients with mucormycosis, 2001 to 2009. *Antimicrob. Agents Chemother.* 2011; **55**: 5018–21.

37 Chamilos G, Lewis RE, Kontoyiannis DP. Delaying amphotericin B-based frontline therapy significantly increases mortality among patients with hematologic malignancy who have zygomycosis. *Clin. Infect. Dis.* 2008; **47**: 503–9.

38 Ibrahim AS, Edwards JE Jr, Filler SG *et al.* Mucormycosis and entomophthoramycosis (zygomycosis). In: Kauffman CA, Pappas PG, Sobel JD *et al.* (eds) *Essentials of Clinical Mycology*, 2nd edn. Springer, New York, 2011; 265–80.

39 Bialek R, Konrad F, Kern J *et al.* PCR-based identification and discrimination of agents of mucormycosis and aspergillosis in paraffin wax embedded tissue. *J. Clin. Pathol.* 2005; **58**: 1180–4.

40 Hammond SP, Bialek R, Milner DA *et al.* Molecular methods to improve the diagnosis and identification of mucormycosis. *J. Clin. Microbiol.* 2011; **49**: 2151–3.

41 Kontoyiannis DP, Lewis R. How I treat mucormycosis. *Blood* 2011; **118**: 1216–24.

42 Lewis RE, Albert ND, Liao G *et al.* Comparative pharmacodynamics of amphotericin B lipid complex and liposomal amphotericin B in a murine model of pulmonary mucormycosis. *Antimicrob. Agents Chemother.* 2010; **54**: 1298–304.

43 Torres HA, Hachem RY, Chemaly RF *et al.* Posaconazole: a broad spectrum triazole agent. *Lancet Infect. Dis.* 2005; **5**: 775–85.

44 Spellberg B, Walsh TJ, Kontoyiannis DP *et al.* Recent advances in the management of mucormycosis: from bench to bedside. *Clin. Infect. Dis.* 2009; **48**: 1743–51.

45 Torres HA, Kontoyiannis DP. Hyalohyphomycoses (hyaline molds). In: Kauffman CA, Pappas PG, Sobel JD *et al.* (eds) *Essentials of Clinical Mycology*, 2nd edn. Springer, New York, 2011; 281–304.

46 Gilgado F, Cano J, Gené J *et al.* Different virulence of the species of the *Pseudallescheria boydii* complex. *Med. Mycol.* 2009; **47**: 371–4.

47 Rodriquez-Tudela JL, Berenguer J, Guarro J *et al*. Epidemiology and outcome of *Scedosporium prolificans* infection: a review of 162 cases. *Med. Mycol.* 2009; **47**: 359–70.

48 Husain S, Alexander B, Munoz P *et al*. Opportunistic mycelial fungi in organ transplant recipients: emerging importance of non-*Aspergillus* mycelial infections. *Clin. Infect. Dis.* 2003; **37**: 221–9.

49 Nucci M, Marr KA, Queiroz-Telles F *et al*. *Fusarium* infection in hematopoietic stem cell transplant recipients. *Clin. Infect. Dis.* 2004; **38**: 1237–42.

50 Nucci M, Anaissie E. *Fusarium* infections in immunocompromised patients. *Clin. Microbiol. Rev.* 2007; **20**: 697–704.

51 Nucci M, Anaissie E. Cutaneous infection by *Fusarium* species in healthy and immunocompromised hosts: implications for diagnosis and management. *Clin. Infect. Dis.* 2002; **35**: 909–20.

52 Castelli MV, Buitrago MJ, Bernal-Martinez L *et al*. Development and validation of a quantitative PCR assay for diagnosis of scedosporiosis. *J. Clin. Microbiol.* 2008; **46**: 3412–6.

53 Hayden RT, Isotalo PA, Parrett T *et al*. *In situ* hybridization for the differentiation of *Aspergillus, Fusarium*, and *Pseudallescheri*a species in tissue section. *Diagn. Mol. Pathol.* 2003; **12**: 21–6.

54 Lortholary O, Obenga G, Biswas P *et al*. International retrospective analysis of 73 cases of invasive fusariosis treated with voriconazole. *Antimicrob. Agents Chemother.* 2010; **54**: 4446–50.

55 Raad II, Hachem RY, Herbrecht R *et al*. Posaconazole as salvage treatment for invasive fusariosis in patients with underlying hematologic malignancy and other conditions. *Clin. Infect. Dis.* 2006; **42**: 1398–403.

56 Ho DY, Lee JD, Rosso F *et al*. Treating disseminated fusariosis: amphotericin B, voriconazole, or both? *Mycoses* 2007; **50**: 227–31.

57 Troke P, Aguirrebengoa K, Arteaga C *et al*. Treatment of scedosporiosis with voriconazole: clinical experience with 107 patients. *Antimicrob. Agents Chemother.* 2008; **52**: 1743–50.

58 Steinbach WJ, Perfect JR. *Scedosporium* species infections and treatments. *J. Chemother.* 2003; **15**: 16–27.

59 Harris JR, Lockhart SR, Debess E *et al*. *Cryptococcus gattii* in the United States: clinical aspects of infection with an emerging pathogen. *Clin. Infect. Dis.* 2011; **53**: 1188–95.

60 Pappas PG, Perfect JR, Cloud GA *et al*. Cryptococcosis in human immunodeficiency virus-negative patients in the era of effective azole therapy. *Clin. Infect. Dis.* 2001; **33**: 690–9.

61 Singh N, Dromer F, Perfect JR *et al*. Cryptococcosis in solid organ transplant recipients: current state of the science. *Clin. Infect. Dis.* 2011; **47**: 1321–7.

62 Chang WC, Tzao C, Hsu HH *et al*. Pulmonary cryptococcosis: comparison of clinical and radiographic characteristics in immunocompetent and immunocompromised patients. *Chest* 2006; **129**: 333–40.

63 Baddley JW, Perfect JR, Oster RA *et al*. Pulmonary cryptococcosis in patients without HV infection: factors associated with disseminated disease. *Eur. J. Clin. Microbiol. Infect. Dis.* 2008; **27**: 937–43.

64 Singh N, Forrest G, Infectious AST. Diseases Community of Practice. Cryptococcosis in solid organ transplant recipients. *Am. J. Transplant.* 2009; **9**(Suppl. 4): S192–8.

65 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.

66 Saccente M, Woods GL. Clinical and laboratory update on blastomycosis. *Clin. Microbiol. Rev.* 2010; **23**: 367–81.

67 Smith JA, Kauffman CA. Blastomycosis. *Proc. Am. Thorac. Soc.* 2010; **7**: 173–80.

68 Lemos LB, Baliga M, Guo M. Acute respiratory distress syndrome and blastomycosis: presentation of nine cases and review of the literature. *Ann. Diagn. Pathol.* 2001; **5**: 1–9.

69 Martynowicz MA, Prakash UB. Pulmonary blastomycosis: an appraisal of diagnostic techniques. *Chest* 2002; **121**: 768–73.

70 Durkin M, Witt J, Lemonte A *et al*. Antigen assay with the potential to aid in diagnosis of blastomycosis. *J. Clin. Microbiol.* 2004; **42**: 4873–5.

71 Bariola JR, Hage CA, Durkin M *et al*. Detection of *Blastomyces dermatitidis* antigen in patients with newly diagnosed blastomycosis. *Diagn. Microbiol. Infect. Dis.* 2011; **69**: 187–91.

72 Carlos WG, Rose AS, Wheat LJ *et al*. Blastomycosis in Indiana: digging up more cases. *Chest* 2010; **138**: 1377–82.

73 Babady NE, Buckwalter SP, Hall L *et al*. Detection of *Blastomyces dermatitidis* and *Histoplasma capsulatum* from culture isolates and clinical specimens by use of real-time PCR. *J. Clin. Microbiol.* 2011; **49**: 3204–8.

74 Chapman SW, Dismukes WE, Proia LA *et al*. Clinical practice guidelines for the management of blastomycosis: 2008 update by the Infectious Diseases Society of America. *Clin. Infect. Dis.* 2008; **46**: 1801–12.

75 Lahm T, Neese S, Thornburg AT *et al*. Corticosteroids for blastomycosis-induced ARDS; a report of two patients and review of the literature. *Chest* 2008; **133**: 1478–80.

76 Kauffman CA. Histoplasmosis: clinical and laboratory update. *Clin. Microbiol. Rev.* 2007; **20**: 115–32.

77 Wheat LJ, Conces D, Allen SD *et al*. Pulmonary histoplasmosis syndromes: recognition, diagnosis, and management. *Sem. Respir. Crit. Care Med.* 2004; **25**: 129–44.

78 Wheat LJ. Approach to the diagnosis of the endemic mycoses. *Clin. Chest Med.* 2009; **30**: 379–89.

79 Swartzentruber S, Rhodes L, Kurkjian K *et al*. Diagnosis of acute pulmonary histoplasmosis by antigen detection. *Clin. Infect. Dis.* 2009; **49**: 1878–82.

80 Hage CA, Davis TE, Fuller D *et al*. Diagnosis of histoplasmosis by antigen detection in BAL fluid. *Chest* 2010; **137**: 623–8.

81 Hage CA, Ribes JA, Wengenack NL *et al*. A multicenter evaluation of tests for diagnosis of histoplasmosis. *Clin. Infect. Dis.* 2011; **53**: 448–54.

82 Wheat LJ, Freifeld AG, Kleiman MB *et al*. Clinical practice guidelines for the management of patients with histoplasmosis: 2007 update by the Infectious Diseases Society of America. *Clin. Infect. Dis.* 2007; **45**: 807–25.

83 Freifeld A, Proia L, Andes D *et al*. Voriconazole use for endemic fungal infections. *Antimicrob. Agents Chemother.* 2009; **53**: 1648–51.

84 Doyle TP, Loyd JE, Robbins IM. Percutaneous pulmonary artery and vein stenting: a novel treatment for mediastinal fibrosis. *Am. J. Respir. Crit. Care Med.* 2001; **164**: 657–60.

85 Ampel NM. Coccidioidomycosis. In: Kauffman CA, Pappas PG, Sobel JD *et al*. (eds) *Essentials of Clinical Mycology*, 2nd edn. Springer, New York, 2011; 349–66.

86 Anstead GM, Graybill JR. Coccidioidomycosis. *Infect. Dis. Clin. North Am.* 2006; **20**: 621–43.

87 Pappagianis D. Current status of serologic studies in coccidioidomycosis. *Curr. Fungal Infect. Rep.* 2007; **1**: 129–34.

88 Durkin M, Connolly P, Kuberski T *et al*. Diagnosis of coccidioidomycosis with use of the *Coccidioides* antigen enzyme immunoassay. *Clin. Infect. Dis.* 2008; **47**: e69–73.

89 Galgiani JN, Ampel NM, Blair JE *et al*. Coccidioidomycosis. *Clin. Infect. Dis.* 2005; **41**: 1217–23.

90 Kim MM, Vikram HR, Kusne S *et al*. Treatment of refractory coccidioidomycosis with voriconazole or posaconazole. *Clin. Infect. Dis.* 2011; **53**: 1060–6.