

Review

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Invasive fungal infections: a review of epidemiology and management options

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Fungi are increasingly recognised as major pathogens in critically ill patients. *Candida* spp. and *Cryptococcus* spp. are the yeasts most frequently isolated in clinical practice. The most frequent filamentous fungi (moulds) isolated are *Aspergillus* spp., but *Fusarium* spp., *Scedosporium* spp., *Penicillium* spp., and Zygomycetes are increasingly seen. Several reasons have been proposed for the increase in invasive fungal infections, including the use of antineoplastic and immunosuppressive agents, broad-spectrum antibiotics, and prosthetic devices and grafts, and more aggressive surgery. Patients with burns, neutropenia, HIV infection and pancreatitis are also predisposed to fungal infection. The epidemiology and clinical features of fungal infections are reviewed, together with antifungal agents currently or soon to be available.

Introduction

Fungi are increasingly recognized as major pathogens in critically ill patients. *Candida* spp. and *Cryptococcus* spp. are the yeasts most frequently isolated in clinical practice. The most frequent filamentous fungi (moulds) isolated are *Aspergillus* spp., but *Fusarium* spp., *Scedosporium* spp., *Penicillium* spp. and Zygomycetes are increasingly seen (Marr *et al.*, 2002; Husain *et al.*, 2003). Several reasons have been proposed for the increase in invasive fungal infections, including the use of antineoplastic and immunosuppressive agents, broad-spectrum antibiotics, and prosthetic devices and grafts, and more aggressive surgery. Patients with burns, neutropenia, HIV infection and pancreatitis are also predisposed to fungal infection (Eggimann *et al.*, 2003).

The epidemiology and clinical features of fungal infections are reviewed, together with antifungal agents currently or soon to be available.

Organisms

Candida spp.

Disseminated candidiasis is associated with a mortality in excess of 25% (Kibbler *et al.*, 2003). *Candida* is a normal commensal of the skin, and gastrointestinal and genitourinary tracts. *Candida albicans* is the most frequent species isolated from clinical specimens, but other species (non-*albicans Candida*, NAC) are increasingly seen. NAC are of special concern, since some are highly virulent and are associated with treatment failure due to reduced susceptibility to antifungal agents. Although identification to species level and susceptibility testing are technically demanding, it is recommended that they should be performed on all fungi obtained from sterile sites and urine of intensive-care and transplant patients (Denning *et al.*, 2003).

Candidaemia rates increased rapidly in the 1980s, so that *Candida* spp. became the fourth-commonest cause of bloodstream infection (BSI) in the USA (Edmond *et al.*, 1999). It has been noted, however, that this increasing trend changed in the late 1990s as a result of a significant decrease in the incidence of *C. albicans* BSI (Trick *et al.*, 2002). The incidence of NAC BSI remained stable, apart from that due to *Candida glabrata*, which increased significantly. This rise in *C. glabrata* also occurred in two prospective studies from Italy (Tortorano *et al.*, 2002, 2004a). Prophylaxis with azole antifungal agents may account for this change in epidemiology. *C. albicans* was responsible for 79.4% of candidaemias in intensive-care patients, but only 37.5% in haematology patients (Kibbler *et al.*, 2003). Similar results were seen in a prospective pan-European study (Tortorano *et al.*, 2004b).

Prior surgery, acute renal failure, previous yeast colonization, neutropenia, antibacterial agents, parenteral nutrition and central venous catheters were associated with an increased risk of invasive candidiasis, whilst prior antifungals was protective (Eggimann *et al.*, 2003).

Candida is capable of causing a wide spectrum of disease. Clinical diagnosis of invasive candida infection is challenging: as most symptoms are non-specific, early clinical manifestations are those of sepsis, and cultures may only become positive late in the course of the infection. Late or no treatment are independent predictors of death in invasive candidiasis (Denning *et al.*, 2003).

Cryptococcus spp.

Cryptococcus neoformans is the commonest cause of cryptococcosis, and is usually acquired by inhalation. Pulmonary cryptococcosis may be asymptomatic or may present

non-specifically with cough, fever or pleural symptoms. Meningitis is a common feature of infection, especially in HIV-seropositive patients and solid-organ transplant recipients.

A high index of suspicion of cryptococcal infection is needed because of the non-specific clinical findings. A positive serum cryptococcal antigen is reliable for the diagnosis of disseminated disease, and should prompt a lumbar puncture to exclude central nervous system disease, as treatment of cryptococcal meningitis requires more aggressive and prolonged therapy (Saag *et al.*, 2000). All cerebrospinal fluid (CSF) specimens from HIV-seropositive patients, transplant recipients and patients with sarcoidosis that show abnormal biochemical parameters or raised leukocytes without adequate explanation should be tested for cryptococcal antigen and cultures incubated for a prolonged period to encourage isolation of the organism (for example, bacterial cultures kept for a minimum of 5 days and fungal cultures for 21 days) (Denning *et al.*, 2003).

***Aspergillus* spp.**

Aspergillus spp. are the most commonly isolated invasive moulds (Denning, 1998). Only a few of the 200 or so species are pathogenic to man, primarily *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger*.

A. fumigatus remains the mould most frequently isolated, but the epidemiology appears to be changing. *A. fumigatus* accounted for 82 % of cases of invasive aspergillosis in 1985, compared to 66 % in 1999 in stem cell transplant patients (Marr *et al.*, 2002). *Aspergillus terreus* is increasingly recognized as a pathogen, accounting for 15 % of isolates in 2001, compared to <2 % in 1996 in one study (Baddley *et al.*, 2003). *Aspergillus* spp. can lead to invasive aspergillosis, tracheobronchitis, aspergilloma and chronic necrotising aspergillosis, but colonization without infection can occur.

Risk factors for invasive aspergillosis include prolonged neutropenia (particularly if >3 weeks) or neutrophil dysfunction, steroid therapy, haematological malignancy, cytotoxic drugs, AIDS, and transplantation (particularly when a mismatch is present). The risk is pronounced in bone marrow transplantation, where invasive aspergillosis is recorded in up to 13 % of allograft and autograft recipients (Marr *et al.*, 2002). Infliximab, an immunosuppressive monoclonal antibody to TNF- α , was recently implicated as a predisposing factor (De Rosa *et al.*, 2003).

Amongst solid-organ transplant patients, lung and heart-lung transplant recipients are at greatest risk of infection, which affects 14–18 % of patients (Hagerty *et al.*, 2003). The transplanted lung is at greatest risk, because the respiratory tract is the primary portal of entry, and the lung is denervated below the anastomosis, leading to loss of cough reflexes and mucociliary clearance. Inadequate blood flow, episodes of rejection, and cytomegalovirus (CMV) infection are also implicated. Up to 7 % of liver transplant recipients develop invasive aspergillosis (Hagerty *et al.*, 2003). The

diagnosis is typically made between 2 and 4 weeks after transplantation, and is associated with poor allograft function with associated increased immunosuppression/post-operative dialysis. However, more episodes (55 %) now occur after 3 months, in association with CMV infection (Singh *et al.*, 2003). Invasive aspergillosis occurs in <1 % of renal transplant cases (Hagerty *et al.*, 2003).

The clinical presentation of invasive aspergillosis varies by patient group, as does the rate at which the disease progresses. As immunosuppression increases, so the rate of progression increases, and the symptoms of infection become less obvious. Invasive aspergillosis may be asymptomatic in up to one-third of patients, and diagnostic difficulties are compounded by the lack of characteristic symptoms and signs. Early symptoms of invasive pulmonary aspergillosis include cough, fever and haemoptysis. Hypoxaemia is usual, and chest radiography changes are variable, although pneumothorax in a neutropenic patient should prompt the clinician to exclude invasive pulmonary aspergillosis as a cause.

Computerized tomography (CT) scanning can be diagnostic (Denning, 1998) and allows early diagnosis. In one study of CT investigation, the mean time to diagnose invasive pulmonary aspergillosis fell from 7 days to <2 days, leading to reduced mortality (Caillot *et al.*, 1997). It also aids further diagnostic studies such as bronchoscopy and open lung biopsy, which may lead to improved outcomes. Typical CT signs include multiple nodules, the halo-sign (seen early in disease) and the air-crescent sign, though the latter may occur only when the neutrophil count is recovering (Caillot *et al.*, 2001). Bronchoscopy fluids should be examined microscopically for hyphae and cultured on specialized media. Clinical isolates of aspergilli should be speciated (Denning *et al.*, 2003). Whilst culture of bronchial fluid is specific (>95 %), it lacks sensitivity (30–50 %). Open lung biopsy is considered to be the gold standard, but false negatives can still occur (Denning 1998). Combining CT with aspergillus antigen (galactomannan) detection also shows promise in early diagnosis of invasive pulmonary aspergillosis (Becker *et al.*, 2003). A recent study combining galactomannan detection, CT and early bronchoscopy suggested that pre-emptive rather than empirical therapy may be more appropriate (Maertens *et al.*, 2005). Further studies are required.

Post lung transplant, airway colonization is present in 29 % of cases and subsequent tracheobronchitis occurs frequently, affecting 5 %, with the area around the anastomosis most at risk (Mehrad *et al.*, 2001). AIDS patients are also susceptible to tracheobronchitis, including those on effective anti-retroviral therapy.

Cerebral infection may present with headache and focal features (Denning, 1998). Sinusitis can also be due to *Aspergillus* spp., although this is more commonly seen in haematology patients than in solid-organ transplant recipients.

Emerging moulds

Other moulds include *Fusarium* spp., *Scedosporium* spp. (hyalohyphomycoses) and *Rhizopus* spp. (a mucormycosis or zygomycosis). These are capable of causing a wide variety of infections. Most cases of disseminated infection occur in immunocompromised patients, particularly in stem-cell (Marr *et al.*, 2002) and solid-organ (Husain *et al.*, 2003) recipients. Other risk factors include diabetes and renal failure (Ribes *et al.*, 2000).

In haematopoietic stem-cell transplant recipients, the incidence of both *Fusarium* spp. and *Rhizopus* spp. infection doubled in the period 1985–1999, whilst that of *Scedosporium* spp. remained stable (Marr *et al.*, 2002). Overall, these genera accounted for 18 % of all mould infections in this group of patients. Infection with *Scedosporium* spp. was more likely to occur whilst the patient was still neutropenic, whilst infection with Zygomycetes was more likely to occur later, corresponding to episodes of graft versus host disease (Ribes *et al.*, 2000). One consequence of the use of voriconazole in prophylaxis and treatment has been a rise in the incidence of infections due to Zygomycetes (Marty *et al.*, 2004).

A prospective multi-centre study of mould infections in heart transplant and liver transplant recipients found that *Aspergillus* spp. were responsible for 69 % of cases of invasive infections (Husain *et al.*, 2003). *Scedosporium* spp. were responsible for four episodes, *Rhizopus* spp. and other Zygomycetes for three cases, and there were two cases caused by *Fusarium* spp. Seven cases were attributed to other mould infections. These moulds were significantly more likely to disseminate from the primary site of infection than *Aspergillus* spp. and were associated with a higher mortality (61.5 versus 54 % for aspergillosis). Retransplantation, CMV infection, dialysis and disseminated infection were associated with increased mortality.

Antifungal agents

Amphotericin B.

Amphotericin B deoxycholate (AmB-D; Fungizone) is a polyene with a very broad spectrum of activity, including most yeasts and filamentous fungi. Possible exceptions include *Candida lusitanae*, *Candida guilliermondii*, *Scedosporium* spp. and *A. terreus*.

Side effects are common, occurring in 50–90 % of cases, and are principally nephrotoxicity or infusion-related. Other side effects include fevers, chills, nausea and vomiting, and hypotension. Nephrotoxicity is related to the use of other nephrotoxic agents and diuretics, and the dosage and duration of amphotericin B treatment. Sepsis, hypotension and tissue hypoxia are also risk factors in intensive-care-unit patients. Alternate-day dosing, administration by continuous infusion, combination with flucytosine to allow use of lower doses, and the use of lipid-associated products, have all been employed to overcome these side effects. Eighty patients were randomized to receive AmB-D either

over 4 h or continuously over 24 h in one trial (Eriksson *et al.*, 2001). Efficacy was equivalent, but nephrotoxicity and infusion-related side effects were significantly lower in the continuous-infusion cohort. However, dedicating an intravenous line for one drug may not be possible in critically ill patients.

Three lipid-associated amphotericin B products are currently available which may have reduced toxicity compared with conventional amphotericin B. These comprise lipid complexes (ABLC-Abelcet, Zeneus Pharma), cholesteryl sulphate (ABCD-Amphocil, Cambridge Laboratories in Europe, Amphotec, InterMune in the USA), and a liposomal formulation (L-AmB; AmBisome, Gilead Sciences). L-AmB is indicated for the treatment of severe systemic and/or deep mycoses in which toxicity (particularly nephrotoxicity) precludes the use of AmB-D in effective dosages, and for the empirical treatment of presumed fungal infections in febrile neutropenic patients, in whom the fever has failed to respond to broad-spectrum antibiotics and appropriate investigations have failed to define a bacterial or viral cause. L-AmB was found to be as effective as AmB-D in the empirical treatment of neutropenic sepsis, and was associated with fewer side effects (Walsh *et al.*, 1999). ABCD is indicated for the treatment of severe systemic and/or deep mycoses in cases in which toxicity or renal failure precludes the use of AmB-D in effective doses and in cases in which prior systemic antifungal therapy has failed. ABCD has been used successfully in severely neutropenic patients. Abelcet is indicated for the treatment of severe invasive candidiasis. Abelcet is also indicated as second-line therapy for the treatment of severe systemic fungal infections in patients who have not responded to AmB-D or other systemic antifungal agents, in those who have renal impairment or other contraindications to AmB-D, or in patients who have developed amphotericin B nephrotoxicity. Abelcet treatment is indicated as second-line treatment for invasive aspergillosis, cryptococcal meningitis and disseminated cryptococcosis in HIV patients, fusariosis, coccidiomycosis, zygomycosis and blastomycosis. L-AmB given at 5 mg kg⁻¹ demonstrated higher efficacy than AmB-D in a study of patients with invasive aspergillosis (Leenders *et al.* 1998), whilst a meta-analysis suggested that lipid-associated formulations reduced all-cause mortality for invasive fungal infections by almost 30 % compared with AmB-D (Barrett *et al.*, 2003). Higher treatment doses of amphotericin B were tolerated due to a reduction in nephrotoxicity. Other drugs with dose-limiting nephrotoxicity (e.g. ciclosporin) may be better tolerated, thereby improving clinical outcome. The meta-analysis failed to show any advantage in outcome of any one lipid-based formulation over another. However, assuming clinical outcome is related to tolerability, the differences in tolerability between the agents assume greater importance. In comparative studies, adverse events requiring discontinuation of the drug have been least with liposomal L-AmB (12 %), intermediate with ABLC (32 %) and highest with ABCD (41 %), the latter figure being comparable to that of AmB-D (44 %) (White *et al.*,

1998; Wingard *et al.*, 2000). The use of lipid-associated formulations is often restricted, because of expense, to patients at high risk of side effects, those who are intolerant of AmB-D, or those who require high doses for prolonged periods.

Flucytosine

Flucytosine (Ancotil, Valeant) is licensed for use in the treatment of systemic fungal infections caused by sensitive organisms. It has activity against *Candida* spp., *Cryptococcus* spp. and some filamentous fungi. Intrinsic resistance in *Candida* spp. is uncommon (Ostrosky-Zeichner *et al.*, 2003), but flucytosine must be combined with another antifungal agent because of the rapid emergence of resistance when used alone. Drug concentrations must be measured regularly, especially with prolonged use of high doses, or when combined with nephrotoxic agents. Side effects are dose-dependent and often predictable, and are rare if peak levels are kept below 100 mg l⁻¹ (Vermes *et al.*, 2000). Gastrointestinal side effects (nausea and diarrhoea), hepatotoxicity and bone marrow suppression are reversible on discontinuation of the drug. They are associated with peak levels greater than 125 mg l⁻¹ (Vermes *et al.*, 2000). Flucytosine levels are reduced by cytarabine, and caution must be employed if used with other myelosuppressive drugs. The combination of amphotericin B with flucytosine has been shown to be beneficial in randomized controlled trials of patients with cryptococcal meningitis (Bennett *et al.*, 1979) and its use is recommended (Saag *et al.*, 2000). Penetration of the drug into the eye and central nervous system is good. Its use is recommended, in combination, in patients with endophthalmitis, meningitis and endocarditis due to *Candida* spp. (Pappas *et al.*, 2004).

Azoles

Fluconazole

Fluconazole (Diflucan, Pfizer) is available in oral or intravenous formulations and is well tolerated, with fewer drug interactions than other azoles. It is inactive against filamentous fungi. Fluconazole has been shown to be as effective as amphotericin B in the treatment of candidaemia in non-neutropenic patients in two large randomized controlled trials (Rex *et al.*, 1994; Phillips *et al.*, 1997). Approximately 5% of isolates of *C. albicans* were resistant to fluconazole, whilst resistance to fluconazole in isolates of *Candida krusei* and *C. glabrata* was noted in 34% (of 50) and 8% (of 458) isolates, respectively (Ostrosky-Zeichner *et al.*, 2003). No resistance, however, occurred in 23 isolates of *C. glabrata* in another study (Kibbler *et al.*, 2003). Fluconazole may be inappropriate for the empirical treatment of suspected fungal infection in neutropenic patients, because prior exposure, as treatment or prophylaxis, is associated with resistant candidal strains, and because of its lack of activity against moulds (Pappas *et al.*, 2004). It may be useful, however, in selected patients who have not received prior azoles, have no risk factors for or features of invasive aspergillosis, and in whom it is unlikely that an azole-resistant

Candida spp. is responsible for infection (Winston *et al.*, 1999).

Itraconazole

Itraconazole (Sporanox, Janssen-Cilag) has a wider spectrum than fluconazole. It is active against yeasts and moulds, with the exception of *Fusarium* spp., *Scedosporium* spp. and the Zygomycetes (Johnson *et al.*, 1998). Reduced susceptibilities occurred in 18% of bloodstream isolates in the USA (8% of *C. albicans* and 51% of *C. glabrata*) (Ostrosky-Zeichner *et al.*, 2003). It has unpredictable bioavailability in capsule form and therapeutic drug monitoring is recommended (Stevens *et al.*, 2000). The introduction of an intravenous preparation and a well-absorbed oral solution has overcome this problem (Barone *et al.*, 1998; Slain *et al.*, 2001).

Clinical data regarding the efficacy of intravenous itraconazole, and information regarding the treatment of invasive candidiasis and invasive aspergillosis, is limited. Itraconazole has been compared with AmB-D in the empirical treatment of neutropenic sepsis (Boogaerts *et al.*, 2001). Mortality rates were similar, whilst itraconazole was associated with significantly fewer adverse events (5 versus 54%), and significantly less withdrawal because of adverse events (19 versus 38%) and nephrotoxicity (5 versus 24%). Intravenous itraconazole is approved for the treatment of pulmonary and extrapulmonary aspergillosis in patients who are intolerant of or who have infections refractory to amphotericin B (Slain *et al.*, 2001). A small retrospective analysis of haematological patients with invasive aspergillosis reported no difference in outcome for patients treated with either amphotericin B or itraconazole (Denning *et al.*, 1998). It is therefore considered acceptable to use itraconazole (400 mg day⁻¹) as intravenous or oral follow-on therapy after initial amphotericin B for the treatment of invasive aspergillosis (Stevens *et al.*, 2000). Itraconazole has been compared with fluconazole for prophylaxis in patients receiving allogeneic stem-cell transplants. One study showed no difference (Morgenstern *et al.*, 1999), whilst another showed a reduction in invasive mould infections but no change in mortality at 250 days (Marr *et al.*, 2004). Itraconazole was associated with significantly more side effects leading to discontinuation (36 versus 16%). These findings were echoed in a recent meta-analysis (Vardakas *et al.* 2005). Side effects included gastrointestinal symptoms, headache, dizziness, raised hepatic transaminases, menstrual disorders, peripheral neuropathy and allergic reactions. Heart failure has been reported, and the UK Committee on Safety of Medicines has advised caution when prescribing itraconazole to older patients or those with cardiac disease. This is particularly important in patients on high doses for prolonged periods.

Itraconazole is known to have many interactions with other drugs which critically ill and immunosuppressed patients are likely to be receiving. Itraconazole is mainly metabolized through the cytochrome CYP3A4. The anticoagulant effect

of warfarin is enhanced by itraconazole. Plasma levels of tacrolimus, ciclosporin, protease inhibitors, statins, calcium channel blockers, digoxin, quinidine, carbamazepine and pimozone are all increased. Itraconazole decreases plasma concentrations of isoniazid, rifampicin, rifabutin, nevirapine and phenytoin, whilst itraconazole levels can be increased by co-administration of macrolides and protease inhibitors.

Voriconazole

Voriconazole (Vfend, Pfizer) has a spectrum similar to that of itraconazole, but extending to several emerging moulds, including *Fusarium* spp. and *Scedosporium* spp. (Diekema *et al.*, 2003). It has *in vitro* activity against many fluconazole-resistant *Candida* spp. (Pfaller *et al.*, 2003). It is licensed in Europe for the treatment of fluconazole-resistant invasive *Candida* infection. Voriconazole was found to be non-inferior to AmB-D followed by fluconazole in a recent randomized trial of non-neutropenic candidaemia when using a 12-week end point (Kullberg *et al.*, 2005). The success rate at the end of therapy was higher (71%) with the AmB-D/fluconazole arm than the voriconazole arm (66%). Adverse reactions were more common in the AmB-D/fluconazole arm, but treatment discontinuation due to adverse events was higher in the voriconazole arm. No trials have been published comparing voriconazole to L-AmB, fluconazole alone or caspofungin in candidaemia. Voriconazole has been compared with fluconazole in the treatment of oesophageal candidiasis, in which it was as effective but was less well tolerated (Ally *et al.*, 2001). More patients discontinued voriconazole because of laboratory test abnormalities and treatment-related adverse events. Voriconazole is also licensed for the primary treatment of invasive aspergillosis and has been compared with AmB-D (1.0–1.5 mg kg⁻¹ day⁻¹) in a non-blinded trial (Herbrecht *et al.*, 2002). Significantly more patients experienced adverse events with amphotericin B (421 events) than with voriconazole (343 events), but no data were presented for the discontinuation rates. At week 12, the survival rate for the voriconazole arm was 71%, and 58% for the amphotericin B arm in the modified intention to treat population. However, the data to suggest that it is superior to amphotericin B are questionable. There were differences in the duration of the initial, non-blinded treatment between voriconazole and amphotericin B, and data indicating superiority are derived from subgroup analysis (71% of all randomized patients) of a secondary outcome measure (survival). A non-blinded trial has compared voriconazole with liposomal amphotericin B in neutropenic patients who remained febrile despite 96 h of therapy with antibacterial agents (Walsh *et al.*, 2002). The rates of successful outcome did not differ between the two groups (26 versus 30.6%, respectively), and both were equally well tolerated. However, significantly more stopped voriconazole therapy due to lack of efficacy (5.3 versus 1.2%, respectively), principally due to persistent fever. Voriconazole is not approved for empirical treatment of febrile neutropenia because the composite primary end points showing superiority were not reached in the study.

Non-inferiority compared with liposomal amphotericin B was therefore not excluded.

Voriconazole is licensed for the treatment of infections due to *Scedosporium* spp. and *Fusarium* spp. on the basis of several case reports. It is ineffective *in vitro* against isolates of Zygomycetes (Sun *et al.*, 2002), and its use may be associated with increased isolation rates of these organisms (Marty *et al.*, 2004). Increased MICs have been seen in an isolate from a patient previously treated with fluconazole (Tortorano *et al.*, 2004b). Although voriconazole has *in vitro* activity against *Cryptococcus* spp., no clinical trials have been published to show superiority over fluconazole, and it remains unlicensed for this indication. Voriconazole is available in oral and intravenous forms. Visual disturbances have been reported in 45% of patients with voriconazole in clinical trials (Herbrecht *et al.*, 2002). Severe reactions, such as Stevens Johnson syndrome, toxic epidermal necrolysis, pancreatitis, hepatitis and jaundice, have been described. Liver function should be monitored before treatment is commenced, and then at 2–4-week intervals. Less-severe side effects include fever, gastrointestinal symptoms, headache and hypotension (Johnson & Kauffman, 2003). Voriconazole is metabolized by cytochrome P450 isoenzymes CYP2C19, CYP2C9 and CYP3A4, and therefore has many drug interactions, similar to those of itraconazole.

Ravuconazole and posaconazole

Ravuconazole and posaconazole have a spectrum of activity similar to that of voriconazole, and are currently undergoing phase III trials. Posaconazole is available only in an oral preparation, but its use has been described in case reports in the treatment of *Scedosporium* spp. (Mellinghoff *et al.*, 2002) and zygomycosis (Tobon *et al.*, 2003). In *in vitro* studies, the activity of posaconazole and ravuconazole was equivalent to that of voriconazole and amphotericin B against *Candida* spp. (Laverdiere *et al.*, 2002; Pfaller *et al.*, 2003) and moulds (Diekema *et al.*, 2003). Posaconazole had more activity *in vitro* against Zygomycetes than voriconazole (Sun *et al.*, 2002), and was found to be more active than amphotericin B against *Scedosporium* spp. (Carrillo & Guarro, 2001). Reduced susceptibility to posaconazole, developing on treatment in an HIV-infected individual, leading to treatment failure, has already been shown to develop in a clinical isolate of *C. albicans* (Li *et al.*, 2004). Posaconazole has been reviewed recently (Torres *et al.*, 2005).

Echinocandins

Caspofungin

The spectrum of caspofungin (Merck Sharpe & Dohme) is limited to *Candida* spp., *Saccharomyces* spp., *Pneumocystis carinii* and *Aspergillus* spp., including *A. terreus* (Denning, 2003). It has good activity against *C. glabrata*, but *Candida parapsilosis* may respond less readily to treatment (Pappas *et al.*, 2004). Caspofungin is available for intravenous use only.

Caspofungin is licensed for the treatment of invasive candidiasis in non-neutropenic adults. Caspofungin was

as effective as AmB-D in the treatment of candidaemia, but was better tolerated (Mora-Duarte *et al.*, 2002). Caspofungin was as effective as amphotericin B ($0.5 \text{ mg kg}^{-1} \text{ day}^{-1}$) (Villanueva *et al.*, 2001) and fluconazole (200 mg) (Villanueva *et al.*, 2002) in the treatment of patients with oesophageal candidiasis, and was well tolerated. Caspofungin is also licensed for the treatment of invasive aspergillosis in adult patients who are refractory to or intolerant of AmB-D, lipid formulations of amphotericin B and/or itraconazole. Refractoriness is defined as progression of infection or failure to improve after a minimum of 7 days of prior therapeutic doses of effective antifungal therapy. A favourable response was noted in 45% of 83 patients (Maertens *et al.*, 2004). Caspofungin is also licensed for empirical therapy for presumed fungal infections (such as *Candida* or *Aspergillus*) in febrile neutropenic adults. Caspofungin was compared to liposomal amphotericin B in a randomized controlled trial involving over 1000 neutropenic patients, and was found to be as effective, but generally better tolerated, in this group of patients (Walsh *et al.*, 2004). Patients on ciclosporin were excluded from this study.

Side effects are uncommon and usually mild. The most important are fever, phlebitis, rash and chills. Mild elevations in liver transaminases and electrolyte disturbances have also been noticed (Mora-Duarte *et al.*, 2002). Headaches have been described (Villanueva *et al.*, 2001, 2002). There are a number of potentially important drug interactions. Rifampicin can reduce caspofungin blood concentrations after prompting an initial rise, whereas ciclosporin may cause a sustained rise in caspofungin levels and area under the curve. Monitoring liver function is advised. Safety data for dual therapy is limited to animal data and small case reports. Increases in caspofungin clearance have been noted when caspofungin is given with carbamazepine, dexamethasone, efavirenz, nelfinavir, nevirapine and phenytoin, suggesting modest dosage increases in caspofungin may be advisable (Denning, 2003). Caspofungin can cause blood concentrations of tacrolimus to fall, and monitoring of tacrolimus levels is advised.

Anidulafungin and micafungin are two new echinocandin agents under development for the treatment of *Candida* spp. Open-label trials have been published with micafungin (Ostrosky-Zeichner *et al.*, 2005), but randomized trials are required.

Combination therapy

Potential benefits of combination therapy include enhanced potency of antifungal efficacy, reduced selection of resistant organisms (particularly for flucytosine), and reduced toxicities due to lower dosing. Evidence of benefit is clearest for the treatment of cryptococcal meningitis, in which the combination of AmB-D and flucytosine leads to earlier sterilization of the CSF (Bennett *et al.*, 1979) and reduced relapse rates (Saag *et al.*, 1999). *In vitro* antagonism of anti-candidal activity has been reported for amphotericin B and azoles, and this combination was not recommended for the

treatment of candidal infections (British Society for Antimicrobial Chemotherapy Working Party, 1994). However, no antagonism was noted clinically in a randomized and blinded trial comparing amphotericin B ($0.7 \text{ mg kg}^{-1} \text{ day}^{-1}$) plus fluconazole (800 mg day^{-1}) with amphotericin B alone (Rex *et al.*, 2003) for the treatment of candidaemia in non-neutropenic patients. Amphotericin B plus fluconazole showed faster bloodstream sterilization than amphotericin B alone.

Despite the high mortality of invasive aspergillosis, there are no published trials of combination therapy for its treatment. In case reports, AmB-D has been combined with flucytosine with occasional success (Darras-Joly *et al.*, 1996). The combination of azoles with amphotericin B, however, is often used. No prospective studies examine the crossover effect from amphotericin B to itraconazole, despite the sequential use of these agents being in the recommendations for the treatment of aspergillosis (Stevens *et al.*, 2000). The sequential use of an azole followed by amphotericin B has also not been studied extensively. A review of published *in vitro* and *in vivo* interactions and 6281 clinical cases revealed only 18 patients treated with itraconazole followed by amphotericin B, with generally poor results (in some instances antagonism was demonstrated) and no standardization (Steinbach *et al.*, 2003). *In vitro* studies of caspofungin with azoles or amphotericin B have generally shown indifference or synergy. Clinical data are limited at present to case reports of patients with invasive aspergillosis. Caspofungin has been used with itraconazole in the treatment of a patient with acute lymphoblastic leukaemia who had invasive pulmonary aspergillosis due to *A. terreus*. The patient remained free of *Aspergillus* infection for more than 7 months, but died of her underlying condition. In another case at the same institution, a lung transplant recipient with aspergillosis due to *A. fumigatus* did not tolerate itraconazole combined with ABLC (5 mg kg^{-1} daily), and so the ABLC was switched to caspofungin, which was well tolerated, the renal function returning to normal (Rubin *et al.*, 2002). In an uncontrolled retrospective report of 48 patients, caspofungin was added to liposomal amphotericin B as salvage therapy in cases of invasive aspergillosis refractory to the latter agent. The combination was well tolerated (Kontoyiannis *et al.*, 2003).

Prophylaxis

The use of fluconazole prophylaxis (400 mg day^{-1}) is commonplace in haematology units, and has been shown to reduce the incidence and severity of fungal infections (Goodman *et al.*, 1992; Slavin *et al.*, 1995) when compared to placebo in bone marrow transplant patients. Meta-analysis has shown improved mortality in stem-cell transplant recipients and patients treated for acute leukaemia (Cornely *et al.*, 2003). Long-term follow-up of stem-cell transplant patients revealed that the persistent protection (i.e. beyond the period of engraftment) in those given fluconazole was beneficial (Marr *et al.*, 2000). Itraconazole (2.5 mg kg^{-1} twice daily) has been compared to

fluconazole in this group of patients and found to be comparable in preventing candidal infection but more effective in preventing mould infection (Morgenstern *et al.*, 1999). A recent trial, however, showed itraconazole to be more toxic and less well tolerated than fluconazole, with no improvement in mortality (Marr *et al.*, 2004). The duration of prophylaxis is currently unknown, but it has been recommended that this should cover the period of neutropenia (Pappas *et al.*, 2004).

Antifungal prophylaxis in the intensive-care setting has been studied extensively, with mixed results. The most recent guidelines suggest that fluconazole may be considered in carefully selected patients if high rates of invasive candidiasis persist despite standard infection-control procedures (Pappas *et al.*, 2004). Patients with severe acute pancreatitis may benefit from early fluconazole prophylaxis (De Waele *et al.*, 2003).

The value of prophylaxis against invasive aspergillus infection remains uncertain. Aerosolized amphotericin B (10 mg twice daily) was compared with no prophylaxis in a randomized trial, but no differences in mortality were noted, and it was poorly tolerated (Schwartz *et al.*, 1999). Risk factors for invasive fungal infection in liver transplant patients include retransplantation, raised creatinine, choledochojejunostomy, significant blood transfusion and fungal colonization (Hagerty *et al.*, 2003). The incidence of invasive *Candida* infection in these patients was reduced by prophylactic fluconazole (compared with placebo), but without effect on mortality (Winston *et al.*, 1999).

Oesophageal candidiasis in HIV-seropositive patients and patients with cancer can be prevented with long-term suppressive therapy with fluconazole. Fluconazole (200 mg daily) has been used as maintenance therapy in HIV-seropositive patients who experienced an episode of cryptococcal meningitis. This was compared favourably to itraconazole (200 mg daily). Relapse occurred in 4% of the fluconazole-treated arm and 23% of the itraconazole-treated arm (Saag *et al.*, 1999).

The uncertain prophylactic value of antifungals and the concern surrounding the generation of resistance prompted the Infectious Disease Society of America to recommend against their general use in neutropenic patients with cancer (Hughes *et al.*, 2002). In view of these uncertainties, it should be noted that careful observation of standard infection-control policies, such as hand washing, use of positive pressure and HEPA-filtered airflow, observation of guidelines for catheter insertion and care, and antibiotic control, are all associated with lowering the incidence of fungal infections in critically ill patients (Manuel & Kibbler, 1998).

Adjunctive therapies

Removal of intravascular cannulae is recommended in the treatment of candidaemias whenever possible, as they are the usual portal of entry (Pappas *et al.*, 2004). Overall

mortality from candidaemia following cannula removal was 40%, compared with 86.9% when cannulae were not removed (Kibbler *et al.*, 2003). Evidence for benefit of line removal is strongest in non-neutropenic patients. The gut is a likely source of infection in neutropenic patients. Treatment of endogenous Cushing's syndrome or lowering doses of iatrogenic corticosteroids is associated with improved outcome.

Several recombinant cytokines have been used in patients at risk of fungal infections. Shortening the period of neutropenia shortens the period of greatest risk, and can be achieved by granulocyte-colony stimulating factor (G-CSF) and granulocyte monocyte-colony stimulating factor (GM-CSF). Interferon γ , interleukin (IL)-12 and anti-IL-4 may be protective as these enhance Th-1-dependent immunity, which is also important in host defence against invasive fungal infections (Roilides *et al.*, 1998).

Conclusions

Amphotericin B, fluconazole and itraconazole are the cornerstones of treatment of invasive fungal infections. The toxicity of AmB-D is frequently dose limiting, and this problem can be avoided with lipid-associated formulations. Voriconazole and caspofungin are two drugs recently licensed for the treatment of invasive fungal infections. Voriconazole has a broad spectrum of activity and is available both orally and parenterally, and may be suitable as second-line therapy in selected patients, particularly those infected with unusual organisms resistant to first-line agents. However, there is no convincing evidence that it is superior to amphotericin B or itraconazole in the treatment of invasive candidal or aspergillus infections. Caspofungin is available in intravenous form only and appears to be better tolerated than AmB-D. At present it is used mainly as a second-line agent in patients with life-threatening invasive candidal or aspergillus infections unresponsive to first-line therapy.

These are interesting times for those managing invasive fungal infections. The increasing numbers of increasingly immunosuppressed patients has led to a rising burden of fungal infection, and the widespread use of antifungal prophylaxis is changing the pattern of fungi recovered in favour of resistant organisms. Pharmaceutical companies are responding to this challenge with an increasing stock of antifungal and immunomodulating agents. Further well-designed studies are needed urgently to guide the use of these drugs.

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