Advances and challenges in management of invasive mycoses

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Invasive mycoses pose a major diagnostic and therapeutic challenge. Advances in antifungal agents and diagnostic methods offer the potential for improved outcomes in patients with these infections, which are often lethal. Many fungal pathogens occur almost exclusively in opportunistic settings—in the immunocompromised host—and these infections are the focus of this review. Several areas of ongoing challenge remain, including the emergence of resistant organisms and the absence of reliable markers for early identification of patients at risk of developing invasive fungal disease. This Seminar reviews the changing epidemiology of invasive mycoses, new diagnostic methods, and recent therapeutic options and current management strategies for these opportunistic pathogens.

Changing epidemiology of invasive mycoses

The epidemiology of invasive mycoses indicates an increasing number of infections in immunosuppressed patients—individuals undergoing transplantation of bone marrow, haemopoietic stem cells, or organ transplantations, and those receiving intensive chemotherapy or other immunosuppressive treatments.1–4 Mortality due to invasive mycoses has also continued to increase.5 The aetiology of invasive mycoses has shown a shift from *Candida albicans* to aspergillus and other moulds, perhaps due to in part to effective control of *C. albicans* with azole prophylaxis, particularly with fluconazole.6,7

A survey published by the US Centers for Disease Control and Prevention5 showed an overall rise in mortality due to invasive mycoses over the past two decades. Notably, overall mortality due to *Candida* spp in this epidemiological study significantly decreased in the past decade, perhaps due to improved recognition of these infections in nosocomial settings and earlier initiation of antifungal therapy.8 Mortality due to *Aspergillus* spp steadily increased, rising more than four-fold over that period.3 Other mycoses, such as moulds like *Fusarium* spp and *Scedosporium* spp—for which limited therapeutic options exist—are associated with even higher mortality rates.

Candida

*Candida* is among the leading causes of nosocomial bloodstream infections worldwide.9–10 Although risk factors for invasive candidosis are well known—including candida colonisation, length of hospital stay, abdominal surgery, and use of parenteral nutrition, antibiotics, or central vascular lines—few assessment strategies can predict a population at high risk of infection.11 The presence of candida in biofilms on catheters and other surfaces provides a nidus of infection that is difficult to eradicate, and substantially contributes to antifungal resistance (figure 1).12 Candida is an important cause of sepsis in the intensive care unit: sepsis due to fungal species increased 207% between 1979 and 2000,13 which was the largest increase observed due to any group of organisms. Crude mortality rates for candidaemia have ranged from 30% to 61%, with significant attributable mortality related to candida.8–10,14

In one study,1 the attributable mortality of nosocomial candidaemia was 49%, which was even higher than the 37% attributable mortality rate reported at the same medical centre 15 years earlier.15

The changing epidemiology of nosocomial candidaemia is due to several factors, including the...
widespread use of antifungals, particularly fluconazole. In the past, C albicans was the usual species associated with invasive infection.18 Now, C albicans comprises less than half the isolates of candidaemia in most series worldwide, although individual variation by medical centre and geographic region has been reported.1,17-21 The importance of this shift relates to the predictable susceptibility patterns associated with species of candida, which is at least partly due to the fact that transferable resistance does not occur in fungi. For example, most strains of C albicans remain susceptible to the triazole antifungals, amphotericin B, and the echinocandins22 (table 1). By contrast, Candida krusei is intrinsically resistant to fluconazole and itraconazole but usually susceptible to the newer triazoles and echinocandins. Candida glabrata, which has increased in many medical centres, shows dose-dependent susceptibility for many strains to fluconazole and itraconazole (ie, higher doses of antifungals are needed for response); it shows increased, but probably clinically achievable, minimum inhibitory concentrations with the newer triazoles, although susceptibility breakpoints have yet to be validated for these drugs.23 The echinocandins offer a broad spectrum of activity to most species of candida. Some strains, such as Candida parapsilosis, show higher minimum inhibitory concentrations with the echinocandins—particularly caspofungin—although the clinical importance of that finding is not clear,24 and breakpoints of susceptibility for the echinocandins have not been established.1,17-21 However, resistance in some isolates, including C glabrata, has been reported.18

The reduced incidence of C albicans is reflected by the increase in prevalence of other yeasts.25 Marr and colleagues11 documented a decrease in candidaemia from a rate of 11·7% to only 4·6% in patients who underwent stem cell transplantation and received fluconazole prophylaxis. However, in patients who experienced a breakthrough infection, non-albicans yeasts, as well as two resistant C albicans strains, were detected.7 Underlying conditions also affect the species of candida: patients with solid tumours were more likely to have infection due to C albicans, whereas those with haematological conditions had non-albicans yeasts.15

Thus, underlying host factors and previous antifungal treatment affect colonisation and the species of candida, which are important issues when choosing initial therapy for the management of candidaemia.

Aspergillus
Mortality rates associated with invasive aspergillosis are still extremely high, particularly in the most extensively immunosuppressed patients and those who develop disseminated infection.1,16 Lin and colleagues reviewed 1941 patients with invasive aspergillosis from 50 published studies and reported an overall mortality of 58%, which was even greater in patients undergoing bone marrow transplantation (87% mortality) and in patients with central nervous system or disseminated infection (90%).3

Species of aspergillus other than Aspergillus fumigatus have been increasingly isolated,7 including Aspergillus terreus, a soil-related species that is often resistant to antifungal therapy, including amphotericin B.38,39 The colony colour and morphology of A terreus is distinct for aspergillus, ranging in colour from buff to beige to cinnamon with characteristic microscopic features (figure 2).33,40 This species is generally more susceptible to the echinocandins and the newer azole antifungals, such as voriconazole and posaconazole, which appear to be better therapeutic options.39,41,42

Changes in the epidemiology of invasive aspergillosis have also emerged. Although most patients with invasive aspergillosis have an underlying haematological malignant disease or are undergoing marrow or stem cell transplantation, a substantial proportion of patients have other causes of immunosuppression, including solid organ transplants, advanced AIDS, and treatment with immunosuppressive therapies such as corticosteroids or other newer immunosuppressive agents such as infliximab, which increase the risk for various infections including unusual moulds.1,16,43,44

Patients with prolonged and profound neutropenia (<100 cells/μL) are at high risk for invasive aspergillosis, but changes in chemotherapeutic regimens and the use of growth factors have limited the duration of neutropenia in some patients, as has been seen in recent clinical trials of antifungal therapy in

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Table 1: Typical in-vitro susceptibility of candida species
Seminar

fever associated with neutropenia. In patients undergoing stem cell transplantation, invasive aspergillosis occurs with bimodal peaks: early (<20 days) after transplantation and very late (>100 days).47,48 One of the factors affecting this change is the occurrence of severe acute or chronic graft-versus-host disease requiring corticosteroid therapy.49 Only 31% of the haemopoietic stem cell transplant patients with invasive aspergillosis reported by Wald and colleagues47 were neutropenic. Similar late infection has also been noted in solid organ transplantation, with a median time to diagnosis of 149 days after transplantation.50

The usual route of infection for invasive aspergillosis is inhalation of aspergillus conidia.51 However, several studies have suggested that exposure can occur through inhalation of water aerosols contaminated with aspergillus.52–54 Hospital water has been documented to contain aspergillus (as well as other moulds like Fusarium spp).53,55 This potential novel route of infection has prompted the suggestion that cleaning hospital showers and toilets might reduce exposure of patients to the mould.56 While evidence continues to support the more traditional role of airborne transmission, timing of antifungal prophylaxis and the optimal strategies for prevention are even more complicated than originally thought.57,58

Agents of hyalohyphomycosis

Hyalohyphomycosis is a term for infections due to fungi with hyaline, septate, branched hyphae. This group of opportunistic moulds (which includes aspergillus) includes organisms that have emerged as uncommon but important pathogens in severely immunocompromised hosts. Numerous monilaceous (lightly-pigmented) moulds have been reported in invasive infection, including Fusarium spp, Scedosporium apiospermum, and species of paecilomyces, trichoderma, acremonium, scopulariopsis, arthrographis, chaetomium, and schizophyllum.4,57 The tissue presentation for most of these organisms mimics aspergillosis, which cannot be definitively distinguished without a culture. The individual organisms are identified by both their macroscopic and microscopic morphologies, especially by their methods of conidiogenesis—the process by which they produce conidia.59

Fusarium spp are the most common of these hyaline moulds, which are resistant to many antifungal agents, including amphotericin B, and mortality rates associated with fusarium species are high—50–80%. Improved susceptibility is seen with the newer triazoles. Encouraging outcomes with voriconazole suggest the newer triazoles are likely to become the drugs of choice for this resistant pathogen.42 Fusarium spp have been documented in hospital water supplies, suggesting a possible water-associated mode of transmission.59,60 Paronychia and skin infections can lead to invasive disease; careful attention to seemingly minor lesions in immunosuppressed patients may reduce the incidence of very serious infection.61

Scedosporium apiospermum is the lightly-pigmented asexual tissue form of Pseudallescheria boydii, an ascomycete which produces dark, round sexual structures in culture. In tissue, hyaline septate hyphae that resemble aspergillosis are seen. In immuno-competent hosts, this organism is a major cause of fungal mycetoma, but in immunosuppressed hosts, widely disseminated infection occurs including the brain.62 This organism may also be resistant to amphotericin B, with activity of voriconazole and the newer azoles shown in both adults and children.42,63

Other hyaline moulds have also emerged as causes of infection in severely immunosuppressed hosts or in specific epidemiological settings. For example, the uncommon mould Phialamonium curvatum has been associated with hospital-acquired infection and an unusual occurrence of community-acquired infection from contaminated intracavernous penile injections.64,65 Diagnosis of these mycoses typically requires classic mycological identification of the organism grown from tissue samples, but investigational PCR and immunohistochemical methods are occasionally useful when cultures are negative. Cultures of blood may be positive in as many as 50% of patients with disseminated fusariosis, which is an important feature of infection due to fusarium.66

Agents of phaeohyphomycosis

Black moulds are a diverse group of organisms characterised by septate, dematiaceous, branched or unbranched fungal elements in tissue that may appear moniliform, beadlike, or swollen.66,67 They include Scedosporium prolificans, exophiala, bipolaris, alternaria,
curvularia, wangiella, and many others. The Masson-Fontana stain is useful to demonstrate melanin in the fungal cell wall, and on culture the organisms appear gray-black and woolly in texture (figure 3). Infections include pulmonary disease, brain lesions, sinusitis, mycetoma, and disseminated disease. Although blood cultures are not positive as frequently as infections due to fusarium, fungaemia can occur with some of these moulds.

Many of the black moulds are susceptible to the extended spectrum azoles, but one resistant pathogen is Scedosporium prolificans. This darkly pigmented organism (previously known as Scedosporium inflatum) is distinguished from the usually lightly pigmented Scedosporium apiospermum by the absence of sexual structures and the characteristic inflated conidiophore in the former. S prolificans causes infections of bone and soft tissue as well as disseminated disease. Outcomes are extremely poor even with the new antifungals drugs, often prompting attempts at combination therapy or local debridement.

**Zygomycosis**

Zygomycosis (also known as mucormycosis) has recently received increased interest because of reports of these infections in severely immunosuppressed patients, particularly those on long-term prophylaxis with voriconazole. Zygomycetes typically cause a syndrome of vascular invasion, thrombosis, and necrosis, which often presents as rhinocerebral infection but also as pulmonary or disseminated disease. The organisms causing this syndrome are in the order Mucorales, although Mucor spp are uncommon causes of infection; genera include rhizopus, absidia, cunninghamamella, Apophysomyces elegans, and Saksenea vasiformis (figure 4). The organisms form broad ribbon-like hyphae in tissue and are generally regarded as non-septate, although rare septae may occur. These organisms may fail to grow from homogenised tissue, which can be a clue to their diagnosis. The organisms are identified by fruiting structures and presence and location of rhizoids—root-like structures along the hyphae.

Historically these infections have occurred in patients with diabetes or burns, but recently their occurrence in other settings has also been noted, such as after trauma or in surgical wounds (figure 4) or, as previously mentioned, in individuals given long-term antifungal prophylaxis.

The emergence of zygomycosis in association with voriconazole therapy has been the focus of particular interest. Because fungal infections occur at a very high rate (30% of more) in high-risk patients, some centres began to use voriconazole as prophylaxis or suppressive therapy. Even though this strategy might reduce the incidence of aspergillosis, breakthrough infections with zygomycetes have been reported by centres where these infections had been uncommon. While the connection between voriconazole therapy and zygomycetes infections has yet to be proven in prospective studies, their simultaneous occurrence worldwide suggests an association, which may also occur with other agents that lack specific activity against zygomycetes, such as the echinocandins. In my opinion, there are several important considerations: breakthrough infections will occur in the highest risk patients and will most likely be resistant to ongoing therapy. The occurrence of an infection on prophylaxis or suppressive therapy with voriconazole (and perhaps other antifungal agents) should raise suspicion of an unusual infection so that extensive efforts should be undertaken to establish the correct diagnosis.

Zygomycoses remain very difficult to treat. Fulminant infections, such as rhinocerebral zygomycosis, require aggressive antifungal therapy, management of underlying disease and surgical debridement. The organisms are resistant to currently available azoles, although posaconazole has activity against many strains and has been effective in salvage therapy.
Amphotericin B (particularly lipid formulations) has been the only other antifungal with demonstrated efficacy, with improved outcomes suggested with higher doses of therapy.81,84

**Recent advances in diagnosis**

The diagnosis of invasive mycoses remains a great challenge. Cultures are frequently not positive and invasive tissue biopsies are reluctantly undertaken in highly immunosuppressed (often pancytopenic) patients. Early treatment is critical to improved outcomes, so that recent treatment has focused on radiography and non-culture-based methods. Despite advances in these alternative methods for the diagnosis of aspergillosis, little success has been seen for other opportunistic mycoses, including candida, for which non-culture-based methods remain largely investigational.

**Radiology**

Radiographic findings are important for the diagnosis of invasive mould infections, particularly invasive pulmonary aspergillosis.85–87 While plain chest radiographs are insensitive and non-specific, CT of the chest can be very useful for establishing a diagnosis, as the halo of low attenuation surrounding a nodular lesion is an early finding in invasive pulmonary aspergillosis.83,87,88 The volume of lesions may increase over the first 7 days of infection, even when therapy is successful, so that early radiological progression should be interpreted with caution.87 An air-crescent is also suggestive of invasive aspergillosis but it is a late finding. These CT findings have been validated in patients with neutropenia or undergoing bone marrow transplant. However, in other patients, such as those who have undergone solid organ transplantation, other agents such as nocardia, other opportunistic pathogens, and non-infectious causes such as pulmonary embolism can be associated with similar findings.

**Non-culture-based methods for aspergillosis**

Non-culture-based methods in aspergillosis have focused on detection of galactomannan (a common cell-wall antigen), PCR, and more recently detection of cell-wall antigens.92 The sensitivity is low—perhaps around 40% or less—in patients with single samples tested, suggesting that serial sampling should be used in high-risk patients.95

The low sensitivity has resulted in recommendations for a lower cutoff for a positive result: an index of 0·5 in the USA, or 0·7–0·8 in Europe.95,96 This lower threshold seems to favourably affect clinical usefulness but assessment of false-positive results is continuing.97 The occurrence of false positives in children and neonates might be due to dietary intake or even to cross-reacting cell-wall motifs from bacteria such as *Bifidobacterium* spp, which heavily colonise the gut and may translocate.98 False positives can also occur in patients who are receiving antibiotic treatment with piperacillin and tazobactam, probably due to galactomannan in the antibiotic preparation.99–104 Other fungi (such as *Penicillium* spp) may produce false positive results due to cross-reacting antigens.95

Although the method has been used with other body fluids, such as cerebrospinal fluid and bronchioalveolar lavage (BAL) fluid, these samples have been less extensively assessed.105–107 Use of this assay for early diagnosis with BAL fluid is particularly intriguing: this approach seems more sensitive and may increase detection rates as much as 30%.106 Despite its potential value, several aspects of the enzyme immunoassay remain uncertain, including the frequency of testing, role of false positives, importance of previous antifungal therapy, and correlation with clinical outcome.95

Other potential markers also include the non-specific fungal marker 1-3,β-D-glucan using a variation of the limulus assay, which detects endotoxin. The β-D-glucan assay (Fungitec G test MK, Seikagaku, Tokyo, Japan; Glucatell [Fungitell], Associates of Cape Cod, Falmouth, MA, USA; β-glucan Wako test, Wako Pure Chemical Industries, Tokyo, Japan; and others), is commercially available in Japan, where it was originally developed and has been used extensively, and is now available worldwide, with recent approval for diagnostic purposes in the USA. These are colorimetric or kinetic assays that can indirectly determine the concentration of 1-3,β-D-glucan in serum samples.108 The test seems promising as an indicator of many fungi, including aspergillus and candida, but not cryptococcus or zygomycetes, which contain little or no β-D-glucan.109

One study suggested that the assay could be useful in early diagnosis of invasive fungal infection in a leukaemic population, but validation remains limited.99 Several reports have shown the potential for using PCR as an early diagnostic marker for invasive aspergillosis.110–113 Most recent studies have used quantitative PCR methods (LightCycler or TaqMan) to detect as little as 10–100 fg of genomic DNA, which correlates with <10 to 100 conidial equivalents per mL.114 The targets detected by these assays include the 18S ribosomal subunit, multi-gene copy mitochondrial.
DNA, and several different single-copy target genes. Assays have been used to detect fungal fragments in blood, plasma, and other body fluids (BAL fluid) to increase early detection.122,123 Sensitivity of most methods varies according to the number of samples taken and extent of disease, but in recent prospective comparative studies sensitivity has generally been around 50–70%.111,116,117 False-positive results due to contamination are less likely with improved PCR techniques, but positive results in patients without apparent disease still occur.116 PCR may be more useful as a very sensitive assay for excluding patients at risk for disease.117 However, necrosis seems to be required for release of fungal DNA, which would limit the use of the assay for early diagnosis.118 A major barrier to the use of PCR is the lack of standardisation.

Which non-culture-based test is the most useful in a clinical setting? Several recent clinical and experimental studies have addressed this question. In an animal model, quantitative galactomannan was somewhat better than PCR in diagnosing and monitoring invasive pulmonary aspergillosis,128 with similar findings in a retrospective clinical study.110 Kawazu and colleagues117 prospectively evaluated 96 patients with haematological disorders at risk for invasive aspergillosis with weekly screening tests with the β-glucan test, PCR, and galactomannan. The galactomannan test performed better than the other methods, but the best results occurred with a combination of PCR and galactomannan testing. Most recently, a combination of the β-glucan test and galactomannan showed a positive predictive value of 100% using both tests in combination.119

In summary, non-culture-based diagnostics have improved the early diagnosis of aspergillosis, although every method has limitations. The tests are most useful when serial assessments are done in high-risk patients, and diagnostic yield may be increased when the tests are used in combination.

Non-culture-based methods for candida and other pathogens
Antigen assays for histoplasma and cryptococcus are well established,120 but, by contrast with aspergillus, non-culture based methods for other opportunistic pathogens remain largely investigational or of limited clinical use. Assays to detect metabolites of candida (such as arabinitol) may complement blood cultures for deep infection, but have limited sensitivity and poor specificity, and are not widely available.121 Although assays for detecting candida antigens are commercially available, they are unable to distinguish colonisation from invasive disease, so that their use is not generally recommended.122 However, one recent study used detection of antibodies to candida cell-wall mannan combined with ELISA assays for candida antigen in an effort to increase the clinical usefulness of the test.122

Advances in antifungal therapy
Amphotericin B deoxycholate has for more than 4 decades been the gold standard treatment for critically ill patients with invasive mycoses.126 Recent studies have consistently documented the limited efficacy and substantial toxicity of amphotericin B deoxycholate in high-risk patients.1,2,127,128 In one study,127 renal toxic effects occurred in about 30% of patients receiving amphotericin B deoxycholate, which was associated with a six-fold increase in mortality and dramatically increased hospital costs. Unacceptably high mortality rates and substantial toxicity have highlighted the need for new therapeutic approaches.129 New antifungal therapies with activity against systemic mycoses have been developed including lipid formulations of amphotericin B, newer azoles, and a new class of antifungal therapy, the echinocandins (table 2).

Lipid formulations of amphotericin and alternative delivery strategies
Lipid formulations of amphotericin B are less toxic but as effective as the parent compound.112,121,124 These agents are particularly useful in refractory infections, as high doses can be administered.113,114 They have largely—and appropriately, in my opinion—replaced amphotericin B deoxycholate, but high cost and potential toxicity of larger doses limit their use. Aerosolised lipid amphotericin B has been used for prophylaxis in lung transplantation.125 An alternative (and less costly from a drug acquisition perspective) approach has used 24-h infusions of standard amphotericin B deoxycholate to decrease toxicity.118,119,121

Echinocandins
The echinocandins are a new class of antifungals with activity against candida and aspergillus but not cryptococcus, zygomycetes, and other moulds.144,146,152–154 These intravenous agents target glucan synthase, which is needed for production of β-1,3-glucan in fungal cell walls.153 They are fungicidal against candida but not aspergillus.113 Current echinocandins include caspofungin, micafungin, and anidulafungin. Caspofungin is approved for patients refractory to or intolerant of standard therapies for invasive aspergillosis and for primary therapy of candida infections, as well as empirical therapy for fever with neutropenia. In salvage therapy of invasive aspergillosis, caspofungin had satisfactory responses in 22 of 54 (41%) patients—including some in whom several other antifungal agents were ineffective.146 Additionally, a large randomised, double-blind study comparing caspofungin to amphotericin B for candidaemia and serious candida infections showed similar efficacy with substantially decreased toxicity.14 Caspofungin is now recommended as a first-line treatment option for candidaemia.21 It is well tolerated with few drug interactions. Interactions with ciclosporin was associated with liver function
abnormalities in normal volunteers although its clinical significance appears minimal.136 Micafungin is approved in Japan and has recently received regulatory approval in the USA for oesophageal candidosis and candida infections with C krusei and C glabrata, and more recently Scedosporium apiospermum, and more recently. Voriconazole was successful in 52% of patients compared with 31% randomised to amphotericin B. A survival benefit of voriconazole was also shown.85 Other studies in adults and children who were refractory to or intolerant of conventional antifungal therapy have confirmed activity in invasive aspergillosis, including responses in central neurological system infection.84,85,138,139 Efficacy of voriconazole against candida has been shown in refractory infection as well as in candida oesophagitis and a large randomised trial of candidaemia.139,140 In this trial,83 voriconazole was equivalent to and less toxic than a regimen of initial amphotericin B followed by fluconazole. A major advantage is that voriconazole can be given orally for resistant yeasts, for convenience of administration and reduction in costs.

Voriconazole has been adequately tolerated and exhibits a favorable pharmacokinetic profile. However, there are a number of issues to consider, including drug intolerance and drug interactions, especially those with immunosuppressive agents such as ciclosporin, tacrolimus, and sirolimus—the latter of which is contra-indicated. The most common adverse event is a transient and reversible visual disturbance, which has been reported in about 30% of patients.80,81 This effect is dose related, and is described as an altered or increased light perception that is temporary and not associated with pathologic sequelae. Other adverse events have been less common, but include raised liver enzyme in 10–15% of patients, skin rash in 6%, nausea and vomiting in 2%, and anorexia in 1%.82,83

### Extended-spectrum triazoles

Another approach has been in the development of extended spectrum triazoles, with new agents including voriconazole, posaconazole, and ravuconazole. Itraconazole is approved for use as salvage therapy of aspergillosis, but its use has been limited due to erratic bioavailability—which requires measurement of drug levels—and drug interactions. An intravenous formulation of itraconazole is available, although there are few efficacy data.86 Itraconazole is effective for prophylaxis and empirical therapy,138,139 but potential toxicity with cyclophosphamide and intolerance has restricted its use.138

Voriconazole is an extended spectrum triazole that is approved for therapy of invasive aspergillosis, Fusarium spp and Scedosporium apiospermum, and more recently candida oesophagitis and candidaemia, including infections with C krusei and C glabrata. Voriconazole has become the recommended primary therapy for most patients with invasive aspergillosis,86 based on a randomised trial that compared voriconazole to amphotericin B deoxycholate, with each agent followed by other licensed antifungal therapy if needed for intolerance or progression.85 Voriconazole was successful in 52% of patients compared with 31% randomised to amphotericin B. A survival benefit of voriconazole was also shown.85 Other studies in adults and children who were refractory to or intolerant of conventional antifungal therapy have confirmed activity in invasive aspergillosis, including responses in central nervous system infection.84,85,138,139 Efficacy of voriconazole against candida has been shown in refractory infection as well as in candida oesophagitis and a large randomised trial of candidaemia.139,140 In this trial,83 voriconazole was equivalent to and less toxic than a regimen of initial amphotericin B followed by fluconazole. A major advantage is that voriconazole can be given orally for resistant yeasts, for convenience of administration and reduction in costs.

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Other extended-spectrum triazoles, including posaconazole and ravuconazole, were developed to include aspergillus.24 Posaconazole is available in only an oral formulation but has been shown to have activity against aspergillus in vitro and in vivo, and in clinical studies.24,140,141 In an open-label trial for salvage therapy of invasive mycoses, posaconazole had activity against invasive aspergillusiosis and other opportunistic pathogens.8,141 A potential advantage of posaconazole is its activity against some Zygomycetes, with favourable clinical results.80 Ravuconazole has been assessed in early phase clinical trials, and has activity in animal models of invasive aspergillusiosis, but is not currently undergoing further clinical development.142,143

### Combination antifungal therapy

The availability of several antifungal drugs and drug classes has increased interest in combination antifungal therapy (and has greatly increased the number of possibilities; table 3).167-168 In an animal model, antagonism occurred between the imidazoles and amphotericin due to alteration of ergosterol in the cell membrane by imidazoles.174 Other experimental models have not shown antagonism with the newer generation azoles, and clinical evidence for antagonism is limited.166,175,176

The use of combinations is most established in the treatment of cryptococcosis, in which initial treatment with amphotericin B with flucytosine is a recommended regimen.165 Even in that disease the possibility of using additional agents, such as fluconazole, has been considered, based on data from animal models. Using a clever approach of rate of change in quantitative CSF cultures, Brouwer and colleagues127 showed that two drug combinations (amphotericin B and flucytosine) produced the best results.

For candida, recent interest has focused on the combination of amphotericin B and higher doses of fluconazole (800 mg/day). A study by Rex and colleagues125 showed improved clearance of the organism from blood with combination therapy compared with fluconazole alone. The usefulness of adding fluconazole to echinocandins is not clear, but was antagonistic in a biofilm model.178

The most interest in combination therapy currently is in invasive aspergillusiosis. Combinations attempted in the past included amphotericin B and rifampicin, flucytosine and others (such as terbinafine).179,180 Unfortunately, in-vitro studies have little use in guiding clinical combination therapy.181 Rifampicin increases metabolism of the azoles, so the combination of rifampicin and azoles is not recommended. Findings in animal models have shown the potential for echinocandins with amphotericin B or with the new azoles in reducing tissue burden and in sterilising tissues, although clinical studies with combination antifungal therapies are limited.143,154,160,187 A recent non-randomised clinical study comparing voriconazole alone or in combination with caspofungin for salvage therapy showed better survival for patients treated with the combination regimen.173 From these results, a randomised clinical trial is warranted to evaluate whether combination antifungal therapy should be used for primary treatment of invasive aspergillusiosis.173,181 In my experience, combination therapy seems to be useful in some patients, but I generally reserve such treatment for patients with progressive infection or disseminated disease.

### Approaches to treatment

Although it is recognised that antifungals must be begun promptly if treatment is likely to be successful, the diagnosis of invasive mycoses remains very difficult. Thus, the clinician is faced with the dilemma of not starting therapy until infection is proven—in which case, outcomes are likely to be poor—or beginning therapy on early suspicion of infection, with the understanding that some patients will receive unnecessary treatment. Since diagnostic markers are still of limited usefulness, clinical suspicion and assessment of risk are required to establish the likelihood of a fungal diagnosis. Early antifungal therapy is likely to improve clinical outcomes and should be targeted at high-risk patients. This approach will allow optimum doses of drugs to be used in the critical early days of treatment, and will avoid the complications and disadvantages of treating large numbers of patients who are unlikely to have a fungal infection. These approaches, together with use of new antifungals, could improve the outcome of patients with these potentially lethal infections.

### Table 3: Antifungal combinations

<table>
<thead>
<tr>
<th>Combination</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>AmB (or LFAB) plus Fluconazole</td>
<td>Improved rate of tissue clearance in cryptococcosis—suggestion of optimum treatment regimen;166 combination may be useful in candida infection for increased efficacy and reduced resistance.167</td>
</tr>
<tr>
<td>Rifampin</td>
<td>Synergistic in vitro; not generally advocated because of drug interactions and induction of hepatic enzymes, which greatly increase the metabolism of newer triazoles166</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>Similar outcome to high doses of fluconazole alone, increased clearance of candidaemia166</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>Potential for antagonism; variable in-vitro effects;166 no antagonism observed in sequential use following initial amphotericin B1</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>Theoretical potential for antagonism; no antagonism demonstrated in experimental infections or in clinical studies166,169</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Echinocandin plus</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>AmB/LFAB plus</td>
<td>In vitro and experimental synergy against yeasts and moulds; anecdotal clinical success for invasive aspergillosis.174-176</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>In-vitro antagonism in a candida biofilm model175</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>Improved clearance of aspergillus in experimental aspergillosis;71 clinical anecdotal success172</td>
</tr>
<tr>
<td>Ravuconazole</td>
<td>Improved efficacy in experimental invasive pulmonary aspergillosis175</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>Improved tissue clearance in experimental aspergillosis;71 anecdotal clinical benefit; better survival in one non-randomised clinical study175</td>
</tr>
</tbody>
</table>

AmB=amphotericin B deoxycholate. LFAB=lipid formulations of amphotericin B.
Conflict of interest statement
I have been a consultant, received grant support or honoraria, and/or have lectures for Pfizer, Merck, Astellas Pharma US, Schering-Pfizer, Bristol-Myers Squibb, Microbio, J Uriach & Cía SA, Vicuron, ENZON, Basilea, Affinium Pharmaceuticals, Rib-X Pharmaceuticals, Diversa, Daiichi, and Nektar Therapeutics.

Acknowledgments
I gratefully acknowledge the support of the National Institutes of Health (grant R01-DE-11381 and contract N01-AI-30041). I have not received any funding for this article except for a small payment from The Lancet.

References
Seminar


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Seminar


