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.

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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 Prevention[5] 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.[5] 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 blood stream infections worldwide.[3,8–10] 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

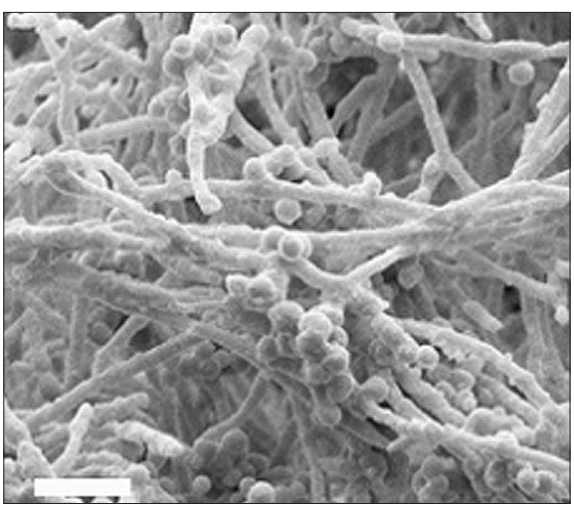

Figure 1: **Scanning electron micrograph of a mature (48 h) *C albicans* biofilm formed on acrylic**
Bar is 20 μm. Courtesy of Jose Lopez-Ribot and Gordon Ramage.

significant attributable mortality related to candida.[8–10,14] In one study,[8] 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

Search strategy and selection criteria

Databases including PubMed (MEDLINE), Current Contents, and the Cochrane Library were searched with the terms "invasive mycoses", "fungal infections", "antifungal", "mycoses", "candidiasis", "aspergillosis", "zygomycosis", "galactomannan", "amphotericin", "triazole", and "echninocandin" and crossed with "epidemiology", "diagnosis", and "therapy". References were largely selected from the last 5 years; older references from my files were used for historical perspective or relevance. Additionally, abstracts from recent major meetings were included to capture recent important advances, particularly in diagnosis and therapy.

Species	Fluconazole	Itraconazole	Voriconazole	Amphotericin	Posaconazole	Candins
C albicans	S	S	S	S	S	S
C tropicalis	S	S	S	S	S	S
C parapsilosis	S	S	S	S	S	S (to I?)
C dubliniensis	S to S-DD	S	S	S	S	S
C glabrata	S-DD to R	S-DD to R	S to I	S to I	S to I	S
C krusei	R	S-DD to R	S to I	S to I	S to I	S
C lusitaniae	S	S	S	S to R	S	S

S=susceptible. R=resistant. S-DD=susceptible dose-dependent. I=intermediate. R=resistant. Formal breakpoints have not been validated for the newer triazoles or echinocandins, and assessments of susceptibility are based on likely achievable serum concentrations of drug, clinical responses, or both. Source: references 17, 22–30.

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

widespread use of antifungals, particularly fluconazole. In the past, *C albicans* was the usual species associated with invasive infection.[16] 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.[3,17–21] 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 echinocandins[22] (table 1[17,22–30]). 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.[31] 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,[23] and breakpoints of susceptibility for the echinocandins have not been established.[14,23,32] However, resistance in some isolates, including *C glabrata*, has been reported.[33]

The reduced incidence of *C albicans* is reflected by the increase in prevalence of other yeasts.[34] Marr and colleagues[7] 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.[35] 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.[1,36] 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%).[2]

Species of aspergillus other than *Aspergillus fumigatus* have been increasingly isolated,[37] 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).[40,41] 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.[24,42,43]

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.[1,44,45]

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

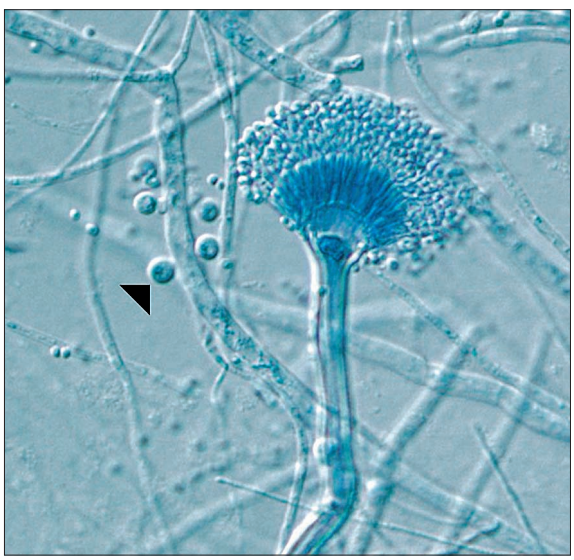

Figure 2: **Photomicrograph of *A terreus***
Columnar, biseriate conidiophore with smooth conidia. Globose, sessile accessory conidia along hyphae (arrow). Original magnification ×420. Courtesy of Deanna Sutton.

fever associated with neutropenia.[46] 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 colleagues[47] 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.[51,54]

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 moniliaceous (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.[58]

Fusarium species 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 immunocompetent 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 *Phialemonium 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.[40,58] They include *Scedosporium prolificans,* exophiala, bipolaris, alternaria,

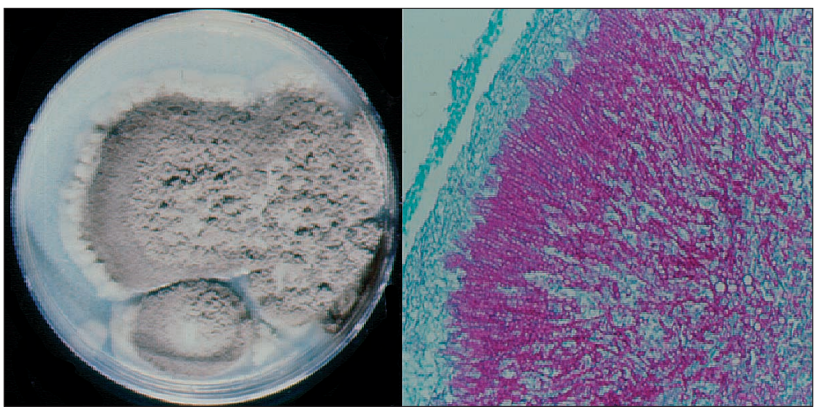

Figure 3: Bipolaris spicifera
Left: colony showing typical gray/black colour and woolly character. Right: Masson-Fontana stain showing melanin in cell wall of mycelia. Courtesy of Deanna Sutton.

curvularia, wangiella, and many others.[67] 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).[40] Infections include pulmonary disease, brain lesions, sinusitis, mycetoma, and disseminated disease.[67] Although blood cultures are not positive as frequently as infections due to fusarium, fungaemia can occur with some of these moulds.[68]

Many of the black moulds are susceptible to the extended spectrum azoles, but one resistant pathogen is *Scedosporium prolificans*.[42,67] 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.[67] Outcomes are extremely poor even with the new antifungals drugs, often prompting attempts at combination therapy or local debridement.[69]

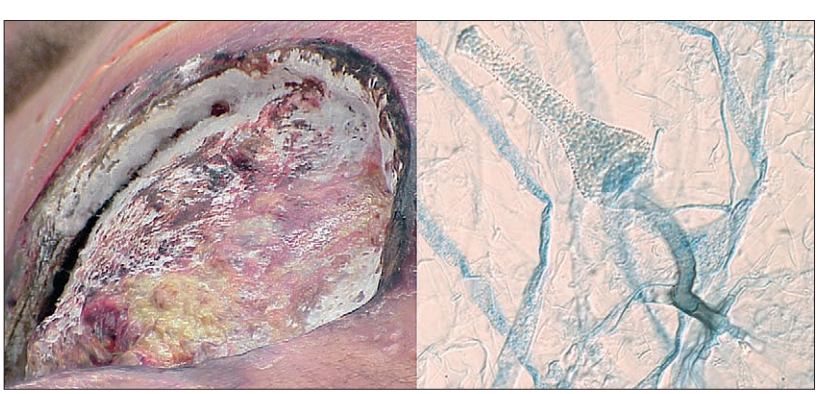

Figure 4: Sakesenea vasiformis
Left: surgical wound infection (courtesy of Daniel Dent and Ronald Stewart). Right: flask-shaped sporangia with swollen bases and long necks, filled with sporangiospores, and branched rhizoids occurring beneath and to the sides of sporangiophores. Courtesy of Deanna Sutton.

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.[70–77] 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, cunninghamella, *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.[40] 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.[74,77,78]

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,[49] some centres began to use voriconazole as prophylaxis or suppressive therapy.[74-77] Even though this strategy might reduce the incidence of aspergillosis,[76] breakthrough infections with zygomycetes have been reported by centres where these infections had been uncommon.[71,73–77]

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.[74,79] 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.[75] 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.[80]

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 efforts have 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.[85,87,88] 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 glucan. An enzyme immunoassay that uses a monoclonal antibody to aspergillus galactomannan is licensed for the diagnosis of invasive aspergillosis. This assay (Platelia *Aspergillus* EIA, Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France; BioRad, Redmond, Washington, USA) has been used most extensively in Europe, and was introduced in the USA in 2003.[89,90] This method lowers the detection limit to 0·5–1·0 ng/mL of galactomannan in serum.[91] The sensitivity for detection of invasive aspergillosis was initially reported at more than 90% with a specificity of greater than 95%.[90,92] Antifungal prophylaxis or empirical therapy reduces the level of circulating galactomannan with reported sensitivity of 30–50%.[93,94] Additionally, 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.[89,96] 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 most extensively used, 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.[109]

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.[112,115] Sensitivity of most methods vary 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.[118] However, necrosis seems to be required for release of fungal DNA, which would limit the use of the assay for early diagnosis.[119] 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,[120] with similar findings in a retrospective clinical study.[110] Kawazu and colleagues[117] 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.[121]

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,[122] 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.[123] 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.[124] 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.[125]

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.[131,132,148] These agents are particularly useful in refractory infections, as high doses can be administered.[81,84] 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.[149] An alternative (and less costly from a drug acquisition perspective) approach has used 24-h infusions of standard amphotericin B deoxycholate to decrease toxicity.[130,150,151]

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.[155] They are fungicidal against candida but not aspergillus.[155] Current echinocandins include caspofungin, micafungin, and anidulafungin. Capsofungin 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.[145] 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.[22] It is well tolerated with few drug interactions. Interactions with ciclosporin was associated with liver function

	Class	Dose and route of administration	Clinical use and major toxic effects
Amphotericin B deoxycholate (AmB)	Polyene	0·6–1·5 mg/kg per day, intravenous	Previous gold standard for invasive mycoses; effectiveness limited in severe immunosuppression and substantial dose-limiting renal toxicity associated with increased mortality and hospital costs; potential decreased toxicity with 24-h infusions[127-129,130]
Lipid formulations of amphotericin B: L-AmB, ABLC, ABCD	Polyene	3–6 mg/kg, intravenous	Less toxicity than AmB; systemic toxicities: ABCD>>ABLC>L-AmB; higher doses anecdotally more effective; cost is a barrier to extensive use[131-134]
Fluconazole	Triazole	400-800 mg per day, intravenous/oral	Active against *C albicans*; variable activity against other yeasts (especially *C glabrata*); better clearance of candidaemia in combination with AmB; not active against moulds; highly bioavailable and well tolerated[22,135]
Itraconazole	Triazole	400–600 mg per day, oral; 200 mg per day, intravenous	Indicated for second-line aspergillosis therapy and sequential use following initial induction therapy; suspension improves bioavailability; limited efficacy data for intravenous form; renal/liver toxicity from chemotherapy interactions; liver and gastrointestinal toxicity[1,136,137]
Voriconazole	Second generation triazole	6 mg/kg per 12 h 3 2 load then 4 mg/kg per 12 h, intravenous; 200 mg twice daily, oral	Invasive aspergillosis: primary therapy in most patients due to survival advantage compared with AmB; other moulds: *Fusarium* spp, scedosporium; not zygomycetes; active against candida including non-albicans; drug interactions; visual, liver, skin toxicity[42,85,138,139]
Posaconzole	Second generation triazole	Investigational, oral	Activity in salvage therapy for invasive mycoses including aspergillosis and zygomycosis; oral suspension; well-tolerated in early trials; gastrointestinal toxicity[80,140,141]
Ravuconazole	Second generation triazole	Investigation, intravenous/oral	Limited clinical development; long-half life; activity in animal models of invasive aspergillosis[142,143]
Caspofungin	Echinocandin	70 mg intravenous load, then 50 mg per day	Invasive candidiasis: less toxicity than AmB and similar efficacy; aspergillosis: salvage and combination therapy; less activity than other moulds; no activity against zygomycetes or cryptococcus; well-tolerated; potential cyclosporin interaction; rare abnormal liver function abnormalities; only intravenous; expensive[14,144,145]
Micafungin	Echinocandin	50–150 mg per day, intravenous	Regulatory approval for prophylaxis in high risk patients and oesophageal candidosis; investigational use for candidaemia, salvage therapy of aspergillosis alone and in combination; well-tolerated in clinical trials[139]
Anidulafungin	Echinocandin	Investigational (100 mg per day intravenous)	Activity in oesophageal candidosis; well-tolerated in early trials; phase III trials ongoing[146,147]

AmB=amphotericin B deoxycholate. L-AmB=liposomal amphotericin B. ABLC=amphotericin B lipid complex. ABCD=amphotericin B colloidal dispersion.

Table 2: **Antifungal agents for invasive fungal infection**

abnormalities in normal volunteers although its clinical significance appears minimal.[156] Micafungin is approved in Japan and has recently received regulatory approval in the USA for oesophageal candidosis and candida prophylaxis.[157] Anidulafungin appears to have activity and a toxicity profile similar to that of other echinocandins.[147]

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.[136] Itraconazole is effective for prophylaxis and empirical therapy,[158,159] but potential toxicity with cyclophosphamide and intolerance has restricted its use.[137]

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,[160] 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.[42,63,138,161] Efficacy of voriconazole against candida has been shown in refractory infection as well as in candida oesophagitis and a large randomised trial of candidaemia.[139,162,163] In this trial,[163] 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.[46,85,139] 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%.[42,85]

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.[24,140,164] In an open-label trial for salvage therapy of invasive mycoses, posaconazole had activity against invasive aspergillosis and other opportunistic pathogens.[43,141] 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 aspergillosis, 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 colleagues[177] 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 colleagues[135] 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 aspergillosis. Combinations attempted in the past included amphotericin B and rifampin, 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.[143,144,169,182] 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 aspergillosis.[173,183] 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.

	Comment
AmB (or LFAB) plus	
Flucytosine	Improved rate of tissue clearance in cryptococcosis—suggestion of optimum treatment regimen;[165] combination may be useful in candida infection for increased efficacy and reduced resistance[22]
Rifampin	Synergistic in vitro; not generally advocated because of drug interactions and induction of hepatic enzymes, which greatly increase the metabolism of newer triazoles[126]
Fluconazole	Similar outcome to high doses of fluconazole alone; increased clearance of candidaemia[135]
Itraconazole	Potential for antagonism; variable in-vitro effects;[126] no antagonism observed in sequential use following initial amphotericin B[1]
Voriconazole	Theoretical potential for antagonism; no antagonism demonstrated in experimental infections or in clinical studies[126,166]
Echinocandin plus	
AmB/LFAB	In-vitro and experimental synergy against yeasts and moulds; anecdotal clinical success for invasive aspergillosis[167-169]
Fluconazole	In-vitro antagonism in a candida biofilm model[170]
Itraconazole	Improved clearance of aspergillus in experimental aspergillosis;[171] clinical anecdotal success[172]
Ravuconazole	Improved efficacy in experimental invasive pulmonary aspergillosis[143]
Voriconazole	Improved tissue clearance in experimental aspergillosis;[144] anecdotal clinical benefit; better survival in one non-randomised clinical study[173]

AmB=amphotericin B deoxycholate. LFAB=lipid formulations of amphotericin B.

Table 3: **Antifungal combinations**

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.

Conflict of interest statement

I have been a consultant, received grant support or honoraria, and/or have lectured for Pfizer, Merck, Astellas Pharma US, Schering-Plough, Bristol-Myers Squibb, Microbia, J Uriach & Cía SA, Vicuron, ENZON, Basilea, Affinium Pharmaceuticals, Rib-X Pharmaceuticals, Diversa, Daiichi, and Nektar Therapeutics.

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References

1 Patterson TF, Kirkpatrick WR, White M, et al. Invasive aspergillosis. Disease spectrum, treatment practices, and outcomes. *Medicine (Baltimore)* 2000; **79:** 250–60.

2 Lin SJ, Schranz J, Teutsch SM. Aspergillosis case-fatality rate: systematic review of the literature. *Clin Infect Dis* 2001; **32:** 358–66.

3 Pappas PG, Rex JH, Lee J, et al. A prospective observational study of candidemia: epidemiology, therapy, and influences on mortality in hospitalized adult and pediatric patients. *Clin Infect Dis* 2003; **37:** 634–43.

4 Marr KA, Carter RA, Crippa F, Wald A, Corey L. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. *Clin Infect Dis* 2002; **34:** 909–17.

5 McNeil MM, Nash SL, Hajjeh RA, et al. Trends in mortality due to invasive mycotic diseases in the United States, 1980–1997. *Clin Infect Dis* 2001; **33:** 641–47.

6 Groll AH, Shah PM, Mentzel C, Schneider M, Just-Nuebling G, Huebner K. Trends in the postmortem epidemiology of invasive fungal infections at a university hospital. *J Infect* 1996; **33:** 23–32.

7 Marr KA, Seidel K, White TC, Bowden RA. Candidemia in allogeneic blood and marrow transplant recipients: Evolution of risk factors after the adoption of prophylactic fluconazole. *J Infect Dis* 2000; **181:** 309–16.

8 Gudlaugsson O, Gillespie S, Lee K, et al. Attributable mortality of nosocomial candidemia, revisited. *Clin Infect Dis* 2003; **37:** 1172–77.

9 Tortorano AM, Biraghi E, Astolfi A, et al. European Confederation of Medical Mycology (ECMM) prospective survey of candidaemia: report from one Italian region. *J Hosp Infect* 2002; **51:** 297–304.

10 Viudes A, Peman J, Canton E, Ubeda P, Lopez-Ribot JL, Gobernado M. Candidemia at a tertiary-care hospital: epidemiology, treatment, clinical outcome and risk factors for death. *Eur J Clin Microbiol Infect Dis* 2002; **21:** 767–74.

11 Ostrosky-Zeichner L. New approaches to the risk of Candida in the intensive care unit. *Curr Opin Infect Dis* 2003; **16:** 533–37.

12 Ramage G, Wickes BL, Lopez-Ribot JL. Biofilms of Candida albicans and their associated resistance to antifungal agents. *Am Clin Lab* 2001; **20:** 42–44.

13 Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med* 2003; **348:** 1546–54.

14 Mora-Duarte J, Betts R, Rotstein C, et al. Comparison of caspofungin and amphotericin B for invasive candidiasis. *N Engl J Med* 2002; **347:** 2020–29.

15 Wey SB, Mori M, Pfaller MA, Woolson RF, Wenzel RP. Hospital acquired candidemia: The attributable mortality and excess length of stay. *Arch Intern Med* 1988; **148:** 2642–45.

16 Edwards JE Jr. Invasive *Candida* infections. Evolution of a fungal pathogen. *N Engl J Med* 1991; **324:** 1060–62.

17 Eggiman P, Garbino J, Pittet D. Epidemiology of *Candida* species infections in critically ill non-immunosuppressed patients. *Lancet Infect Dis* 2003; **3:** 685–702.

18 Pfaller MA, Jones RN, Messer SA, Edmond MB, Wenzel RP. National surveillance of nosocomial blood stream infection due to species of *Candida* other than *Candida albicans*: frequency of occurrence and antifungal susceptibility in the SCOPE program. *Diagn Microbiol Infect Dis* 1998; **30:** 121–29.

19 Pfaller MA, Jones RN, Doern GV, Sader HS, Hollis RJ, Messer SA. International surveillance of bloodstream infections due to *Candida* species: frequency of occurrence and antifungal susceptibilities of isolates collected in 1997 in the United States, Canada, and South America for the SENTRY program. *J Clin Microbiol* 1998; **36:** 1886–89.

20 Pfaller MA, Diekema DJ, Jones RN, et al. International surveillance of bloodstream infections due to *Candida* species: frequency of occurrence and in vitro susceptibilities to fluconazole, ravuconazole, and voriconazole of isolates collected from 1997 through 1999 in the SENTRY antimicrobial surveillance program. *J Clin Microbiol* 2001; **39:** 3254–59.

21 Rangel-Frausto MS, Wiblin T, Blumberg HM, et al. National Epidemiology of Mycoses Survey (NEMIS): variations in rates of bloodstream infections due to *Candida* species in seven surgical intensive care units and six neonatal intensive care units. *Clin Infect Dis* 1999; **29:** 253–58.

22 Pappas PG, Rex JH, Sobel JD, et al. Guidelines for treatment of candidiasis. *Clin Infect Dis* 2004; **38:** 161–89.

23 Ostrosky-Zeichner L, Rex JH, Pappas PG, et al. Antifungal susceptibility survey of 2,000 bloodstream Candida isolates in the United States. *Antimicrob Agents Chemother* 2003; **47:** 3149–54.

24 Pfaller MA, Messer SA, Hollis RJ, Jones RN. Antifungal activities of posaconazole, ravuconazole, and voriconazole compared to those of itraconazole and amphotericin B against 239 clinical isolates of *Aspergillus* spp. and other filamentous fungi: Report from SENTRY Antimicrobial Surveillance Program, 2000. *Antimicrob Agents Chemother* 2002; **46:** 1032–37.

25 Pfaller MA, Diekema DJ, Messer SA, Boyken L, Huynh H, Hollis RJ. Clinical evaluation of a frozen commercially prepared microdilution panel for antifungal susceptibility testing of seven antifungal agents, including the new triazoles posaconazole, ravuconazole, and voriconazole. *J Clin Microbiol* 2002; **40:** 1694–97.

26 Bartizal K, Gill CJ, Abruzzo GK, et al. In vitro preclinical evaluation studies with the echinocandin antifungal MK-0991 (L-743,872). *Antimicrobial Agents Chemother* 1997; **41:** 2326–32.

27 Patterson TF. Role of newer azoles in surgical patients. *J Chemotherapy* 1999; **11:** 504–12.

28 Rex JH, Pfaller MA, Galgiani JN, et al. Development of interpretive breakpoints for antifungal susceptibility testing: conceptual framework and analysis of *in vitro-in vivo* correlation data for fluconazole, itraconazole, and *Candida* infections. *Clin Infect Dis* 1997; **24:** 235–47.

29 Roling EE, Klepser ME, Wasson A, Lewis RE, Ernst EJ, Pfaller MA. Antifungal activities of fluconazole, caspofungin (MK0991), and anidulafungin (LY 303366) alone and in combination against *Candida* spp. and *Crytococcus neoformans* via time-kill methods. *Diagn Microbiol Infect Dis* 2002; **43:** 13–17.

30 Diekema DJ, Messer SA, Brueggemann AB, et al. Epidemiology of candidemia: 3-year results from the emerging infections and the epidemiology of Iowa organisms study. *J Clin Microbiol* 2002; **40:** 1298–302.

31 Pfaller MA, Messer SA, Hollis RJ, Jones RN, Diekema DJ. In vitro activities of ravuconazole and voriconazole compared with those of four approved systemic antifungal agents against 6,970 clinical isolates of Candida spp. *Antimicrob Agents Chemother* 2002; **46:** 1723–27.

32 Pfaller MA, Messer SA, Coffman S. In vitro susceptibilities of clinical yeast isolates to a new echinocandin derivatives, LY303366, and other antifungal agents. *Antimicrob Agents Chemother* 1997; **41:** 763–66.

33 Villarreal NC, Fothergill AW, Kelly C, Patterson JE, Rinaldi MG, Patterson TF. *Candida glabrata* resistance to caspofungin during therapy (M-1034). Abstracts of the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy; Oct 30–Nov 2, 2004; Washington, DC.

34 Abi-Said D, Anaissie E, Uzun O, Raad I, Pinzcowski H, Vartivarian S. The epidemiology of hematogenous candidiasis caused by different *Candida* species. *Clin Infect Dis* 1997; **24:** 1122–28.

35 Viscoli C, Girmenia C, Marinus A, et al. Candidemia in cancer patients: a prospective, multicenter surveillance study by the Invasive Fungal Infection Group (IFIG) of the European Organization for Research and Treatment of Cancer (EORTC). *Clin Infect Dis* 1999; **28:** 1071–79.

36 Denning DW. Early diagnosis of invasive aspergillosis. *Lancet* 2000; **355:** 423–24.

37 Perfect JR, Cox GM, Lee JY, et al. The impact of culture isolation of *Aspergillus* species: a hospital-based survey of aspergillosis. *Clin Infect Dis* 2001; **33:** 1824–33.

38 Iwen PC, Rupp ME, Langnas AN, Reed EC, Hinrichs SH. Invasive pulmonary aspergillosis due to Aspergillus terreus: 12- year experience and review of the literature. *Clin Infect Dis* 1998; **26:** 1092–97.

39 Steinbach WJ, Benjamin DK Jr, Kontoyiannis DP, et al. Infections due to *Aspergillus terreus*: a multicenter retrospective analysis of 83 cases. *Clin Infect Dis* 2004; **39:** 192–98.

40 Sutton DA, Fothergill AW, Rinaldi MG, eds. Guide to clinically significant fungi. 1st edn. Baltimore: Williams and Wilkins, 1998.

41 Walsh TJ, Petraitis V, Petraitiene R, et al. Experimental pulmonary aspergillosis due to *Aspergillus terreus*: pathogenesis and treatment of an emerging fungal pathogen resistant to amphotericin B. *J Infect Dis* 2003; **188:** 305–19.

42 Perfect JR, Marr KA, Walsh TJ, et al. Voriconazole treatment for less-common, emerging, or refractory fungal infections. *Clin Infect Dis* 2003; **36:** 1122–31.

43 Raad I, Chapman S, Bradsher R, et al. Posaconazole (POS) salvage therapy for invasvie fungal infections (IFI) (abstract M-699). Abstracts of the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy; Oct 30–Nov 2, 2004; Washington, DC.

44 Warris A, Bjorneklett A, Gaustad P. Invasive pulmonary aspergillosis associated with infliximab therapy. *N Engl J Med* 2001; **344:** 1099–100.

45 Marty FM, Lee SJ, Fahey MM, et al. Infliximab use in patients with severe graft-versus-host disease and other emerging risk factors of non-Candida invasive fungal infections in allogeneic hematopoietic stem cell transplant recipients: a cohort study. *Blood* 2003; **102:** 2768–76.

46 Walsh TJ, Pappas P, Winston DJ, et al. Voriconazole compared with liposomal amphotericin B for empirical antifungal therapy in patients with neutropenia and persistent fever. *N Engl J Med* 2002; **346:** 225–34.

47 Wald A, Leisenring W, van Burik J-A, Bowden RA. Epidemiology of *Aspergillus* infections in a large cohort of patients undergoing bone marrow transplantation. *J Infect Dis* 1997; **175:** 1459–66.

48 Marr KA, Carter RA, Boeckh M, Martin P, Corey L. Invasive aspergillosis in allogeneic stem cell transplant recipients: changes in epidemiology and risk factors. *Blood* 2002; **100:** 4358–66.

49 Jantunen E, Ruutu P, Niskanen L, et al. Incidence and risk factors for invasive fungal infections in allogenic BMT receipients. *Bone Marrow Transplant* 1997; **19:** 801–08.

50 Pappas P. Prospective surveillance for invasive fungal infections (IFIs) in hematopoetic stem cell (HSCTs) and solid organ transplant recipients (SOTs) in the United States (abstract M-1010). 43rd Annual Interscience Conference on Antimicrobial Agents and Chemotherapy; Sept 14–17, 2003; Chicago, IL: 458.

51 Hajjeh RA, Warnock DW. Counterpoint: invasive aspergillosis and the environment—rethinking our approach to prevention. *Clin Infect Dis* 2001; **33:** 1549–52.

52 Anaissie EJ, Stratton SL, Dignani MC, et al. Pathogenic molds (including Aspergillus species) in hospital water distribution systems: a 3-year prospective study and clinical implications for patients with hematologic malignancies. *Blood* 2003; **101:** 2542–46.

53 Anaissie EJ, Costa SF. Nosocomial aspergillosis is waterborne. *Clin Infect Dis* 2001; **33:** 1546–48.

54 Graybill JR. Aspergillosis: from the breeze or from the bucket? *Clin Infect Dis* 2001; **33:** 1545.

55 Warris A, Voss A, Abrahamsen TG, Verweij PE. Contamination of hospital water with *Aspergillus fumigatus* and other molds. *Clin Infect Dis* 2002; **34:** 1159–60.

56 Anaissie EJ, Owens S, Dignani MC, Lee CK, Rex J, Walsh T. Cleaning bathrooms: a novel approach to reducing patient exposure to aerosolized *Aspergillus* spp. *Blood* 2001; **98:** 207A.

57 Sigler L, Verweij PR. *Aspergillus, Fusarium*, and other opportunistic moniliaceous fungi. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH, eds. Manual of clinical microbiology. 8th edn. Washington, DC: American Society for Microbiology, 2003: 1726–60.

58 Sutton DA. Laboratory evaluation of new antifungal agents against rare and refractory mycoses. *Curr Opin Infect Dis* 2002; **15:** 575–82.

59 Anaissie EJ, Kuchar RT, Rex JH, et al. Fusariosis associated with pathogenic *Fusarium* species colonization of a hospital water system: a new paradigm for the epidemiology of opportunistic mold infections. *Clin Infect Dis* 2001; **33:** 1871–78.

60 Anaissie E, Kuchar R, Rex JH, Summerbell R, Walsh T. The hospital water system as a reservoir *for Fusarium*. 37th Interscience Conference on Antimicrobial Agents and Chemotherapy; 1997; San Diego, USA.

61 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.

62 Castiglioni B, Sutton DA, Rinaldi MG, Fung J, Kusne S. *Pseudallescheria boydii* (Anamorph *Scedosporium apiospermum*). Infection in solid organ transplant recipients in a tertiary medical center and review of the literature. *Medicine (Baltimore)* 2002; **81:** 333–48.

63 Walsh TJ, Lutsar I, Driscoll T, et al. Voriconazole in the treatment of aspergillosis, scedosporiosis and other invasive fungal infections in children. *Pediatr Infect Dis J* 2002; **21:** 240–48.

64 Guarro J, Nucci M, Akiti T, et al. *Phialemonium fungemia*: two documented nosocomial cases. *J Clin Microbiol* 1999; **37:** 2493–97.

65 Strahilevitz J, Rahav G, Schroers HJ, et al. An outbreak of Phialemonium infective endocarditis linked to intracavernous penile injections for the treatment of impotence. *Clin Infect Dis* 2005; **40:** 781–86.

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

67 Revankar SG, Patterson JE, Sutton DA, Pullen R, Rinaldi MG. Disseminated phaeohyphomycosis: Review of an emerging mycosis. *Clin Infect Dis* 2002; **34:** 467–76.

68 Nucci M, Akiti T, Barreiros G, et al. Nosocomial fungemia due to *Exophiala jeanselmei* var. *jeanselmei* and a Rhinocladiella species: newly described causes of bloodstream infection. *J Clin Microbiol* 2001; **39:** 514–18.

69 Steinbach WJ, Schell WA, Miller JL, Perfect JR. *Scedosporium prolificans* osteomyelitis in an immunocompetent child treated with voriconazole and caspofungin, as well as locally applied polyhexamethylene biguanide. *J Clin Microbiol* 2003; **41:** 3981–85.

70 Dannaoui E, Meis JF, Loebenberg D, Verweij PE. Activity of posaconazole in treatment of experimental disseminated zygomycosis. *Antimicrob Agents Chemother* 2003; **47:** 3647–50.

71 Kobayashi K, Kami M, Murashige N, Kishi Y, Fujisaki G, Mitamura T. Breakthrough zygomycosis during voriconazole treatment for invasive aspergillosis. *Haematologica* 2004; **89:** ECR42.

72 Vigouroux S, Morin O, Moreau P, et al. Zygomycosis after prolonged use of voriconazole in immunocompromised patients with hematologic disease: attention required. *Clin Infect Dis* 2005; **40:** e35–37.

73 Oren I. Breakthrough zygomycosis during empirical voriconazole therapy in febrile patients with neutropenia. *Clin Infect Dis* 2005; **40:** 770–71.

74 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:** 1350–59.

75 Imhof A, Balajee SA, Fredricks DN, Englund JA, Marr KA. Breakthrough fungal infections in stem cell transplant recipients receiving voriconazole. *Clin Infect Dis* 2004; **39:** 743–46.

76 Siwek GT, Dodgson KJ, de Magalhaes-Silverman M, et al. Invasive zygomycosis in hematopoietic stem cell transplant recipients receiving voriconazole prophylaxis. *Clin Infect Dis* 2004; **39:** 584–87.

77 Marty FM, Cosimi LA, Baden LR. Breakthrough zygomycosis after voriconazole treatment in recipients of hematopoietic stem-cell transplants. *N Engl J Med* 2004; **350:** 950–52.

78 Andreson D, Donaldson A, Choo L, et al. Multifocal cutaneous mucormycosis complicating polymicrobial wound infections in a tsunami survivor from Sri Lanka. *Lancet* 2005, **365:** 876–78.

79 Kauffman CA. Zygomycosis: reemergence of an old pathogen. *Clin Infect Dis* 2004; **39:** 588–90.

80 Greenberg RN, Anstead G, Herbrecht R, et al. Posaconazole (POS) experience in the treatment of zygomycosis (abstract M-1757). 43rd Annual Interscience Conference on Antimicrobial Agents and Chemotherapy; Sept 14–17, 2003; Chicago, IL: 476.

81 Herbrecht R, Letscher-Bru V, Bowden RA, et al. Treatment of 21 cases of invasive mucormycosis with amphotericin B colloidal dispersion. *Eur J Clin Microbiol Infect D* 2001; **20:** 460-466.

82 Gonzalez CE, Couriel DR, Walsh TJ. Disseminated zygomycosis in a neutropenic patient: Successful treatment with amphotericin B lipid complex and granuloyte colony-stimulating factor. *Clin Infect Dis* 1997; **24:** 192–196.

83 Walsh TJ, Hiemenz JW, Seibel NL, et al. Amphotericin B lipid complex for invasive fungal infections: analysis of safety and efficacy in 556 cases. *Clin Infect Dis* 1998; **26:** 1383–96.

84 Walsh TJ, Goodman JL, Pappas P, et al. Safety, tolerance, and pharmacokinetics of high-dose liposomal amphotericin B (AmBisome) in patients infected with *Aspergillus* species and other filamentous fungi: Maximum tolerated dose study. *Antimicrob Agents Chemother* 2001; **45:** 3487–96.

85 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.

86 Ascioglu S, Rex JH, de Pauw B, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: An international consensus. *Clin Infect Dis* 2002; **34:** 7–14.

87 Caillot D, Couaillier JF, Bernard A, et al. Increasing volume and changing characteristics of invasive pulmonary aspergillosis on sequential thoracic computed tomography scans in patients with neutropenia. *J Clin Oncol* 2001; **19:** 253–59.

88 Greene RE, Schlamm HT, Stark P, et al. Radiological findings in acute invasive pulmonary aspergillosis: utility and reliability of halo sign and air-crescent sign for diagnosis and treatment of IPA in high-risk patients (abstract O397). Program of the 13th European Congress of Clinical Microbiology and Infectious Diseases; May, 2003; Glasgow.

89 US Food and Drug Administration. FDA clears rapid test for *Aspergillus* infection. US Food and Drug Administration, 2003.

90 Maertens J, Verhaegen J, Lagrou K, Van Eldere J, Boogaerts M. Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. *Blood* 2001; **97:** 1604–10.

91 Sulahian A, Tabouret M, Ribaud P, et al. Comparison of an enzyme immunoassay and latex agglutination test for detection of galactomannan in the diagnosis of aspergillosis. *Eur J Clin Microbiol Infect D* 1996; **15:** 139–45.

92 Swanink CMA, Meis JFGM, Rijs AJMM, Donnelly JP, Verweij PE. Specificity of a sandwich enzyme-linked immunosorbent assay for detecting *Aspergillus* galactomannan. *J Clin Microbiol* 1997; **35:** 257–60.

93 Herbrecht R, Letscher-Bru V, Oprea C, et al. Aspergillus galactomannan detection in the diagnosis of invasive aspergillosis in cancer patients. *J Clin Oncol* 2002; **20:** 1898–906.

94 Pinel C, Fricker-Hidalgo H, Lebeau B, et al. Detection of circulating *Aspergillus fumigatus* galactomannan: value and limits of the Platelia test for diagnosing invasive aspergillosis. *J Clin Microbiol* 2003; **41:** 2184–86.

95 Mennink-Kersten MA, Donnelly JP, Verweij PE. Detection of circulating galactomannan for the diagnosis and management of invasive aspergillosis. *Lancet Infect Dis* 2004; **4:** 349–57.

96 Marr KA, Balajee SA, McLaughlin L, Tabouret M, Bentsen C, Walsh TJ. Detection of galactomannan antigenemia by enzyme immunoassay for the diagnosis of invasive aspergillosis: variables that affect performance. *J Infect Dis* 2004; **190:** 641–49.

97 Maertens J, Theunissen K, Verbeken E, et al. Prospective clinical evaluation of lower cut-offs for galactomannan detection in adult neutropenic cancer patients and haematological stem cell transplant recipients. *Br J Haematol* 2004; **126:** 852–60.

98 Mennink-Kersten MA, Klont RR, Warris A, Op den Camp HJ, Verweij PE. Bifidobacterium lipoteichoic acid and false ELISA reactivity in aspergillus antigen detection. *Lancet* 2004; **363:** 325–27.

99 Sulahian A, Touratier S, Ribaud P. False positive test for aspergillus antigenemia related to concomitant administration of piperacillin and tazobactam. *N Engl J Med* 2003; **349:** 2366–67.

100 Viscoli C, Machetti M, Cappellano P, et al. False-positive galactomannan platelia Aspergillus test results for patients receiving piperacillin-tazobactam. *Clin Infect Dis* 2004; **38:** 913–16.

101 Walsh TJ, Shoham S, Petraitiene R, et al. Detection of galactomannan antigenemia in patients receiving piperacillin-tazobactam and correlations between in vitro, in vivo, and clinical properties of the drug-antigen interaction. *J Clin Microbiol* 2004; **42:** 4744–48.

102 Penack O, Schwartz S, Thiel E, Wolfgang Blau I. Lack of evidence that false-positive Aspergillus galactomannan antigen test results are due to treatment with piperacillin-tazobactam. *Clin Infect Dis* 2004; **39:** 1401–02.

103 Mattei D, Rapezzi D, Mordini N, et al. False-positive Aspergillus galactomannan enzyme-linked immunosorbent assay results in vivo during amoxicillin-clavulanic acid treatment. *J Clin Microbiol* 2004; **42:** 5362–63.

104 Mennink-Kersten MAS, Klont RR, Ruegebrink D, Op den Camp H, Verweij PE. Amoxicillin-clavulanic acid (AMC) and piperacillin-tazobactam (PTZ) contain high molecular weight cross reacting aspergillus antigen (abstract M-1684a). Abstracts of the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy; Oct 30–Nov 2, 2004; Washington, DC.

105 Viscoli C, Machetti M, Gazzola P, et al. *Aspergillus* galactomannan antigen in the cerebrospinal fluid of bone marrow transplant recipients with probable cerebral aspergillosis. *J Clin Microbiol* 2002; **40:** 1496–99.

106 Becker MJ, Lugtenburg EJ, Cornelissen JJ, Van Der Schee C, Hoogsteden HC, De Marie S. Galactomannan detection in computerized tomography-based broncho-alveolar lavage fluid and serum in haematological patients at risk for invasive pulmonary aspergillosis. *Br J Haematol* 2003; **121:** 448–57.

107 Klont RR, Mennink-Kersten MA, Verweij PE. Utility of Aspergillus antigen detection in specimens other than serum specimens. *Clin Infect Dis* 2004; **39:** 1467–74.

108 Miyazaki T, Kohno S, Mitsutake K, et al. Plasma (1→3)-beta-D-glucan and fungal antigenemia in patients with candidemia, aspergillosis, and cryptococcosis. *J Clin Microbiol* 1995; **33:** 3115–18.

109 Odabasi Z, Mattiuzzi G, Estey E, et al. Beta-D-glucan as a diagnostic adjunct for invasive fungal infections: validation, cutoff development, and performance in patients with acute myelogenous leukemia and myelodysplastic syndrome. *Clin Infect Dis* 2004; **39:** 199–205.

110 Costa C, Costa JM, Desterke C, Botterel F, Cordonnier C, Bretagne S. Real-time PCR coupled with automated DNA extraction and detection of galactomannan antigen in serum by enzyme-linked immunosorbent assay for diagnosis of invasive aspergillosis. *J Clin Microbiol* 2002; **40:** 2224–27.

111 Costa C, Vidaud D, Olivi M, Bart-Delabesse E, Vidaud M, Bretagne S. Development of two real-time quantitative TaqMan PCR assays to detect circulating *Aspergillus fumigatus* DNA in serum. *J Microbiol Meth* 2001; **44:** 263–69.

112 Loeffler J, Hebart H, Brauchle U, Schumacher U, Einsele H. Comparison between plasma and whole blood specimens for detection of Aspergillus DNA by PCR. *J Clin Microbiol* 2000; **38:** 3830–33.

113 Pham AS, Tarrand JJ, May GS, Lee MS, Kontoyiannis DP, Han XY. Diagnosis of invasive mold infection by real-time quantitative PCR. *Am J Clin Pathol* 2003; **119:** 38–44.

114 Loeffler J, Hebart H, Cox P, Flues N, Schumacher U, Einsele H. Nucleic acid sequence-based amplification of Aspergillus RNA in blood samples. *J Clin Microbiol* 2001; **39:** 1626–29.

115 Musher B, Fredricks D, Leisenring W, Balajee SA, Smith C, Marr KA. Aspergillus galactomannan enzyme immunoassay and quantitative PCR for diagnosis of invasive aspergillosis with bronchoalveolar lavage fluid. *J Clin Microbiol* 2004; **42:** 5517–22.

116 Buchheidt D, Hummel M, Schleiermacher D, et al. Prospective clinical evaluation of a LightCycler-mediated polymerase chain reaction assay, a nested-PCR assay and a galactomannan enzyme-linked immunosorbent assay for detection of invasive aspergillosis in neutropenic cancer patients and haematological stem cell transplant recipients. *Br J Haematol* 2004; **125:** 196–202.

117 Kawazu M, Kanda Y, Nannya Y, et al. Prospective comparison of the diagnostic potential of real-time PCR, double-sandwich enzyme-linked immunosorbent assay for galactomannan, and a (1→3)-beta-D-glucan test in weekly screening for invasive aspergillosis in patients with hematological disorders. *J Clin Microbiol* 2004; **42:** 2733–41.

118 Verweij PE, Klont RR, Donnelly JP. Validating PCR for detecting invasive aspergillosis. *Br J Haematol* 2004; **127:** 235–36.

119 Mennink-Kersten MASH, Ruegebrink D, Klont RR, Melchers WJG, Verweij PE. Fungal DNA is not released by *Aspergillus fumigatus* in an in vitro kinetic model (abstract M-1677). In: Abstracts of the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy; 30 Oct–2 Nov, 2004; Washington, DC.

120 Becker MJ, de Marie S, Willemse D, Verbrugh HA, Bakker-Woudenberg IA. Quantitative galactomannan detection is superior to PCR in diagnosing and monitoring invasive pulmonary aspergillosis in an experimental rat model. *J Clin Microbiol* 2000; **38:** 1434–38.

121 Pazos C, Ponton J, Del Palacio A. Contribution of (1→3)-beta-D-glucan chromogenic assay to diagnosis and therapeutic monitoring of invasive aspergillosis in neutropenic adult patients: a comparison with serial screening for circulating galactomannan. *J Clin Microbiol* 2005; **43:** 299–305.

122 Wheat J. Histoplasma capsulatum antigen detection: Comparison of the performance characteristics of a new inhibition immunoassay to those of an established antibody sandwich immunoassay. *J Clin Microbiol* 1999; **37:** 2387.

123 Walsh TJ, Hathorn JW, Sobel JD, et al. Detection of circulating *Candida* enolase by immunoassay in patients with cancer and invasive candidiasis. *N Engl J Med* 1991; **324:** 1026–31.

124 Verweij PE, Poulain D, Obayashi T, Patterson TF, Denning DW, Ponton J. Current trends in the detection of antigenaemia, metabolites and cell wall markers for the diagnosis and therapeutic monitoring of fungal infections. *Med Mycol* 1998; **36:** 146–55.

125 Sendid B, Poirot JL, Tabouret M, et al. Combined detection of mannanaemia and antimannan antibodies as a strategy for the diagnosis of systemic infection caused by pathogenic *Candida* species. *J Med Microbiol* 2002; **51:** 433–42.

126 Stevens DA, Kan VL, Judson MA, et al. Practice guidelines for diseases caused by *Aspergillus. Clin Infect Dis* 2000; **30:** 696–709.

127 Bates DW, Su L, Yu DT, et al. Mortality and costs of acute renal failure associated with amphotericin B therapy. *Clin Infect Dis* 2001; **32:** 686–93.

128 Wingard JR, Kubilis P, Lee L, et al. Clinical significance of nephrotoxicity in patients treated with amphotericin B for suspected or proven aspergillosis. *Clin Infect Dis* 1999; **29:** 1402–07.

129 Ostrosky-Zeichner L, Marr KA, Rex JH, Cohen SH. Amphotericin B: time for a new "gold standard". *Clin Infect Dis* 2003; **37:** 415–25.

130 Imhof A, Walter RB, Schaffner A. Continuous infusion of escalated doses of amphotericin B deoxycholate: an open-label observational study. *Clin Infect Dis* 2003; **36:** 943–51.

131 Barrett JP, Vardulaki KA, Conlon C, et al. A systematic review of the antifungal effectiveness and tolerability of amphotericin B formulations. *Clin Ther* 2003; **25:** 1295–320.

132 Bowden R, Chandrasekar P, White MH, et al. A double-blind, randomized, controlled trial of amphotericin B colloidal dispersion versus amphotericin B for treatment of invasive aspergillosis in immunocompromised patients. *Clin Infect Dis* 2002; **35:** 359–66.

133 Wingard JR, White MH, Anaissie E, Raffalli J, Goodman J, Arrieta A. A randomized, double-blind comparative trial evaluating the safety of liposomal amphotericin B versus amphotericin B lipid complex in the empirical treatment of febrile neutropenia. *Clin Infect Dis* 2000; **31:** 1155–63.

134 Leenders ACAP, Daenen S, Jansen RLH, et al. Liposomal amphotericin B compared with amphotericin B deoxycholate in the treatment of documented and suspected neutropenia-associated invasive fungal infections. *Br J Haematol* 1998; **103:** 205–12.

135 Rex JH, Pappas PG, Karchmer AW, et al. A randomized and blinded multicenter trial of high-dose fluconazole plus placebo versus fluconazole plus amphotericin B as therapy for candidemia and its consequences in nonneutropenic subjects. *Clin Infect Dis* 2003; **36:** 1221–28.

136 Caillot D, Bassaris H, McGeer A, et al. Intravenous itraconazole followed by oral itraconazole in the treatment of invasive pulmonary aspergillosis in patients with hematologic malignancies, chronic granulomatous disease, or AIDS. *Clin Infect Dis* 2001; **33:** E83–E90.

137 Marr KA, Crippa F, Leisenring W, et al. Itraconazole versus fluconazole for prevention of fungal infections in patients receiving allogeneic stem cell transplants. *Blood* 2004; **103:** 1527–33.

138 Denning DW, Ribaud P, Milpied N, et al. Efficacy and safety of voriconazole in the treatment of acute invasive aspergillosis. *Clin Infect Dis* 2002; **34:** 563–71.

139 Ally R, Schurmann D, Kreisel W, et al. A randomized, double-blind, double-dummy, multicenter trial of voriconazole and fluconazole in the treatment of esophageal candidiasis in immunocompromised patients. *Clin Infect Dis* 2001; **33:** 1447–54.

140 Petraitiene R, Petraitis V, Groll AH, et al. Antifungal activity and pharmacokinetics of posaconazole (SCH 56592) in treatment and prevention of experimental invasive pulmonary aspergillosis: Correlation with galactomannan antigenemia. *Antimicrob Agents Chemother* 2001; **45:** 857–69.

141 Hachem RY, Raad II, Afif CM, et al. An open, noncomparative multicenter study to evaluate efficacy and safety of posaconazole (SCH 56592) in the treatment of invasive fungal infections refractory to or intolerant of standard therapy. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy. Toronto: 2000.

142 Kirkpatrick WR, Perea S, Coco BJ, Patterson TF. Efficacy of ravuconazole (BMS-207147) in a guinea pig model of disseminated aspergillosis. *J Antimicrob Chemother* 2002; **49:** 353–57.

143 Petraitis V, Petraitiene R, Sarafandi AA, et al. Combination therapy in treatment of experimental pulmonary aspergillosis: synergistic interaction between an antifungal triazole and an echinocandin. *J Infect Dis* 2003; **187:** 1834–43.

144 Kirkpatrick WR, Perea S, Coco BJ, Patterson TF. Efficacy of caspofungin alone and in combination with voriconazole in a Guinea pig model of invasive aspergillosis. *Antimicrob Agents Chemother* 2002; **46:** 2564–68.

145 Maertens J, Raad I, Petrikkos G, et al. Efficacy and safety of caspofungin for treatment of invasive aspergillosis in patients refractory to or intolerant of conventional antifungal therapy. *Clin Infect Dis* 2004; **39:** 1563–71.

146 Denning DW. Echinocandin antifungal drugs. *Lancet* 2003; **362:** 1142–51.

147 Krause DS, Reinhardt J, Vazquez JA, et al. Phase 2, randomized, dose-ranging study evaluating the safety and efficacy of anidulafungin in invasive candidiasis and candidemia. *Antimicrob Agents Chemother* 2004; **48:** 2021–24.

148 Linden PK, Coley K, Fontes P, Fung JJ, Kusne S. Invasive aspergillosis in liver transplant recipients: outcome comparison of therapy with amphotericin B lipid complex and a historical cohort treated with conventional amphotericin B. *Clin Infect Dis* 2003; **37:** 17–25.

149 Palmer SM, Drew RH, Whitehouse JD, et al. Safety of aerosolized amphotericin B lipid complex in lung transplant recipients. *Transplantation* 2001; **72:** 545–48.

150 Peleg AY, Woods ML. Continuous and 4 h infusion of amphotericin B: a comparative study involving high-risk haematology patients. *J Antimicrob Chemother* 2004; **54:** 803–08.

151 Andes D, Stamsted T, Conklin R. Pharmacodynamics of amphotericin B in a neutropenic-mouse disseminated-candidiasis model. *Antimicrob Agents Chemother* 2001; **45:** 922–26.

152 Petraitis V, Petraitiene R, Groll AH, et al. Dosage-dependent antifungal efficacy of V-echinocandin (LY303366) against experimental fluconazole-resistant oropharyngeal and esophageal candidiasis. *Antimicrob Agents Chemother* 2001; **45:** 471–79.

153 Petraitiene R, Petraitis V, Groll AH, et al. Antifungal efficacy of caspofungin (MK-0991) in experimental pulmonary aspergillosis in persistently neutropenic rabbits: pharmacokinetics, drug disposition, and relationship to galactomannan antigenemia. *Antimicrob Agents Chemother* 2002; **46:** 12–23.

154 Petraitis V, Petraitiene R, Groll AH, et al. Comparative antifungal activities and plasma pharmacokinetics of micafungin (FK463) against disseminated candidiasis and invasive pulmonary aspergillosis in persistently neutropenic rabbits. *Antimicrob Agents Chemother* 2002; **46:** 1857–69.

155 Bowman JC, Hicks PS, Kurtz MB, et al. The antifungal echinocandin caspofungin ccetate kills growing cells of *Aspergillus fumigatus* in vitro. *Antimicrob Agents Chemother* 2002; **46:** 3001–12.

156 Marr KA, Hachem R, Papanicolaou G, et al. Retrospective study of the hepatic safety profile of patients concomitantly treated with caspofungin and cyclosporin A. *Transpl Infect Dis* 2004; **6:** 110–16.

157 van Burik JA, Ratanatharathorn V, Stepan DE, et al. Micafungin versus fluconazole for prophylaxis against invasive fungal infections during neutropenia in patients undergoing hematopoietic stem cell transplantation. *Clin Infect Dis* 2004; **39:** 1407–16.

158 Boogaerts M, Winston DJ, Bow EJ, et al. Intravenous and oral itraconazole versus intravenous amphotericin B deoxycholate as empirical antifungal therapy for persistent fever in neutropenic patients with cancer who are receiving broad-spectrum antibacterial therapy. A randomized, controlled trial. *Ann Intern Med* 2001; **135:** 412–22.

159 Winston DJ, Maziarz RT, Chandrasekar PH, et al. Intravenous and oral itraconazole versus intravenous and oral fluconazole for long-term antifungal prophylaxis in allogeneic hematopoietic stem-cell transplant recipients. A multicenter, randomized trial. *Ann Intern Med* 2003; **138:** 705–13.

160 Steinbach WJ, Stevens DA. Review of newer antifungal and immunomodulatory strategies for invasive aspergillosis. *Clin Infect Dis* 2003; **37** (suppl 3)**:** S157–87.

161 Troke PF, Schwartz S, Ruhnke M, et al. Voriconazole (VRC) therapy (Rx) in 86 patients (pts) with CNS aspergillosis (CNSA): a retrospective analysis (M-1755). 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy. Chicago: Sept 14–17, 2003: 476.

162 Ostrosky-Zeichner L, Oude Lashof AM, Kullberg BJ, Rex JH. Voriconazole salvage treatment of invasive candidiasis. *Eur J Clin Microbiol Infect Dis* 2003; **22:** 651–55.

163 Kullberg BJ, Pappas P, Ruhnke M, et al. Voriconazole compared with a strategy of amphotericin B followed by fluconazole for treatment of candidaemia in non-neutropenic patients (abstract O245). 14th European Congress of Clinical Microbiology and Infectious Diseases. Prague: 1–4 May, 2004.

164 Kirkpatrick WR, McAtee RK, Fothergill AW, Loebenberg D, Rinaldi MG, Patterson TF. Efficacy of posaconazole in a rabbit model of invasive aspergillosis. *Antimicrob Agents Chemother* 2000; **44:** 780–82.

165 Saag MS, Graybill RJ, Larsen RA, et al. Practice guidelines for the management of cryptococcal disease. *Clin Infect Dis* 2000; **30:** 710–18.

166 Kirkpatrick WR, Coco BJ, Patterson TF. Sequential or combination therapy with voriconazole and liposomal amphotericin B in a guinea pig model of Aspergillosis. Program and Abstracts of Trends in Invasive Fungal Infections 7; Sept 28–Oct 1; Amsterdam; 2003.

167 Kontoyiannis DP, Hachem R, Lewis RE, et al. Efficacy and toxicity of caspofungin in combination with liposomal amphotericin B as primary or salvage treatment of invasive aspergillosis in patients with hematologic malignancies. *Cancer* 2003; **98:** 292–99.

168 Aliff TB, Maslak PG, Jurcic JG, et al. Refractory Aspergillus pneumonia in patients with acute leukemia: successful therapy with combination caspofungin and liposomal amphotericin. *Cancer* 2003; **97:** 1025–32.

169 Kohno S, Maesaki S, Iwakawa J, et al. Synergistis effects of combination of FK463 with amphotericin B: enhanced efficacy in murine model of invasive pulmonary aspergillosis. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy; 2000; Toronto, CA,USA.

170 Bachmann SP, VandeWalle K, Ramage G, et al. In vitro activity of caspofungin against *Candida albicans* biofilms. *Antimicrob Agents Chemother* 2002; **46:** 3591–96.

171 Douglas CM, Abruzzo G, Bowman JC, et al. Caspofungin alone or in combination with itraconazole reduces fungal burden in a neutropenic guinea pig model of disseminated aspergillosis. Abstracts of the 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy; 2002; San Diego, CA, USA: M-1819.

172 Rubin MA, Carroll KC, Cahill BC. Caspofungin in combination with itraconazole for the treatment of invasive aspergillosis in humans. *Clin Infect Dis* 2002; **34:** 1160–61.

173 Marr KA, Boeckh M, Carter RA, Kim HW, Corey L. Combination antifungal therapy for invasive aspergillosis. *Clin Infect Dis* 2004; **39:** 797–802.

174 Schaffner A, Frick PG. The effect of ketoconazole on amphotericin B in a model of disseminated aspergillosis. *J Infect Dis* 1985; **151:** 902–10.

175 Boucher HW, Herbrecht R, Bennett JE, et al. The strategy of following voriconazole (VRC) vs amphotericin B (AMB) with other licensed antifungal therapy (OLAT) for primary therapy of invasive aspergillosis (IA) (abstract M-964). 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, September 14–17, 2003: 446.

176 George D, Kordick D, Miniter P, Patterson TF, Andriole VT. Combination therapy in experimental invasive aspergillosis. *J Infect Dis* 1993; **168:** 692–98.

177 Brouwer AE, Rajanuwong A, Chierakul W, et al. Combination antifungal therapies for HIV-associated cryptococcal meningitis: a randomised trial. *Lancet* 2004; **363:** 1764–67.

178 Bachmann SP, Ramage G, VandeWalle K, Patterson TF, Wickes BL, Lopez-Ribot JL. Antifungal combinations against Candida albicans biofilms in vitro. *Antimicrob Agents Chemother* 2003; **47:** 3657–59.

179 Denning DW, Hanson LH, Perlman AM, Stevens DA. In vitro susceptibility and synergy studies of *Aspergillus* species to conventional and new agents. *Diagn Microbiol Infect Dis* 1992; **15:** 21–34.

180 Denning DW, Stevens DA. Antifungal and surgical treatment of invasive aspergillosis: review of 2,121 published cases. *Rev Infect Dis* 1990; **12:** 1147–201.

181 Steinbach WJ, Stevens DA, Denning DW. Combination and sequential antifungal therapy for invasive aspergillosis: review of published in vitro and in vivo interactions and 6281 clinical cases from 1966 to 2001. *Clin Infect Dis* 2003; **37** (suppl 3)**:** S188–224.

182 Nakajima M, Tamada S, Yoshida K, et al. Pathological findings in a murine pulmonary aspergillosis model: Treatment with FK463, amphotericin B, and a combination of FK463 and amphotericin B. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy; 2000; Toronto, CA, USA.

183 Viscoli C. Combination therapy for invasive aspergillosis. *Clin Infect Dis* 2004; **39:** 803–05.