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Review

Nosocomial fungal infections: epidemiology, diagnosis, and treatment

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Invasive fungal infections are increasingly common in the nosocomial setting. Furthermore, because risk factors for these infections continue to increase in frequency, it is likely that nosocomial fungal infections will continue to increase in frequency in the coming decades. The predominant nosocomial fungal pathogens include *Candida* spp., *Aspergillus* spp., *Mucorales*, *Fusarium* spp., and other molds, including *Scedosporium* spp. These infections are difficult to diagnose and cause high morbidity and mortality despite antifungal therapy. Early initiation of effective antifungal therapy and reversal of underlying host defects remain the cornerstones of treatment for nosocomial fungal infections. In recent years, new antifungal agents have become available, resulting in a change in standard of care for many of these infections. Nevertheless, the mortality of nosocomial fungal infections remains high, and new therapeutic and preventative strategies are needed.

Keywords nosocomial fungal infections, *Candida* spp., *Aspergillus* spp., *Mucorales*, *Fusarium* spp.

Introduction and overview

Over the past several decades, the incidence of nosocomial fungal infections (i.e., invasive fungal infections acquired in a health care-associated setting) has dramatically increased. Factors responsible for the rise of these infections include aging populations in countries with advanced medical technologies, the resultant increase in incidence of many cancers, increasingly intensive myeloablative therapies for these cancers, increasingly intensive care for critically ill patients, and increases in the frequency of solid organ and hematopoietic stem cell transplantation. These demographic trends are anticipated to continue to rise over the coming decades, portending a continued increase in the incidence of invasive fungal infections in the nosocomial setting.

The relative frequencies with which fungi cause nosocomial infections are inversely related to the intensity of immunosuppression required to predispose to them. For example, relatively minimal immunosuppression is required to predispose to invasive *Candida* infections, and *Candida* is by far the most common cause of nosocomial fungal infections [1–7]. *Aspergillus* is the second most frequent cause of nosocomial fungal infections [1–10], and aspergillosis tends to occur in patients with an intermediate to severe degree of immunocompromise. Finally, organisms such as the *Mucorales*, *Fusarium*, and other molds (e.g., *Scedosporium*) are relatively less common, and are seen virtually exclusively in the most severely immunocompromised hosts, and in hosts that are compromised for prolonged periods of time.

A commonality shared by all nosocomial fungal infections is the difficulty in establishing the diagnosis.

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Despite many years of intensive study, few diagnostic studies are available and reliable for these infections. Hence, emphasis has been placed on early empiric therapy in patients who are clinically suspected of having a fungal infection, or on prophylaxis for the highest risk patients.

In contrast to the lack of progress in diagnosis, major advances in the medical therapy of nosocomial fungal infections have been made in recent years, with the introduction and widespread availability of lipid-associated amphotericin products, newer triazole agents, and the newest, echinocandin class of antifungals. Ironically, the availability of an extensive antifungal armamentarium has created new clinical conundrums, including how to prioritize the antifungal options for each type of infection, and, most controversial of all, the role of combination antifungal therapy for these infections.

Based on the above considerations, the purpose of this review is to summarize the current knowledge about the epidemiology, diagnostic testing, and management of nosocomial invasive fungal infections. Although endemic mycoses such as *Coccidioides*, *Histoplasma*, and *Blastomyces* may present in immunocompromised patients in hospital settings, they are rarely acquired in health care-associated settings, and thus will not be discussed.

Candida

Frequency

Candida species are by far the most common fungi causing invasive disease in humans. Data from the National Nosocomial Infection Survey (NNIS) indicated that *Candida* spp. were the fourth most common cause of nosocomial bloodstream infections in the 1990s [11,12]. However, more recent studies have found that *Candida* spp. are now the third most frequent nosocomial bloodstream isolates [13–15], statistically tied with *Enterococcus,* and surpassed in frequency only by *Staphylococcus epidermidis* and *Staphylococcus aureus*. These datasets are limited by the fact that bloodstream infection is defined by blood culture positivity. It is known from older autopsy series as well as more recent clinical investigations that 30–50% of patients with disseminated candidiasis (defined as candidal infection in sterile target organs with or without positive blood cultures) have negative blood cultures [16,17]. Therefore, the true incidence of disseminated candidiasis is markedly underrepresented by datasets focusing on blood cultures.

The under-estimation of the frequency of disseminated candidiasis by datasets based on blood cultures is highlighted by data from population-based studies. The annual US incidence of blood culture-confirmed candidemia reported in such surveys is approximately 10 cases per 100,000 population [18,19]. Since up to half of cases of disseminated candidiasis are missed by blood cultures, it can be estimated that the frequency of disseminated candidiasis is 20 cases per 100,000 population. Indeed, in a study based on billing codes (which reflect clinical diagnoses of disseminated candidiasis, rather than relying upon blood cultures), the frequency of disseminated candidiasis was approximately 24 cases per 100,000 population [1]. Hence an estimated 60–70,000 cases of disseminated candidiasis occur per year in the US alone. Because each of these cases adds tens of thousands of dollars to hospitalization costs, it has been estimated that the health care cost associated with hematogenously disseminated candidiasis is $2–4 billion/year in the US alone [1,20].

The incidence of disseminated candidiasis has increased 15 to 20-fold compared to two decades earlier [21–26]. However, studies reported a leveling off of the frequency of invasive *Candida* infections during the late 1990s [27–30]. Since risk factors for disseminated candidiasis continue to increase in frequency, it is not clear why the incidence of invasive *Candida* infections would not continue to increase. One potential explanation is that the burgeoning use of prophylactic or early empiric azole therapy limits the ability to confirm by culture cases of disseminated candidiasis [28]. Hence, recent data on the frequency of invasive *Candida* infections may underestimate the true incidence of disease. Furthermore, most recently, several studies have been published contradicting the notion that the incidence of disseminated candidiasis is leveling off, and demonstrating a continued rise in its incidence since the turn of the 21st century [28,31,32].

Risk factors

Numerous studies have defined the risk factors that predispose patients to developing invasive *Candida* infections. The impact of these risk factors is significant, as high risk/hospitalized patients have a ~50-fold increase in incidence of disseminated candidiasis compared to patients with fewer risk factors [12,18,33–35].

What is generally underappreciated is that while *Candida* is an opportunistic pathogen, the majority of patients (~80%) who develop disseminated candidiasis are not immunosuppressed in the classical sense (e.g., neutropenic, corticosteroid-treated, infected with HIV, etc.) [18,19,21,34,36–42]. Rather, the predominant risk

Table 1 Major risk factors for invasive *Candida* infections

Iatrogenic/Nosocomial Conditions*	Immunosuppression*
Colonization	Neutropenia
Broad spectrum antibiotics	Corticosteroids
Central venous catheter	HIV‡
Parenteral nutrition	Diabetes mellitus‡
Gastrointestinal or cardiac surgery	
Prolonged hospital stay†	
ICU stay	
Burns	
Premature neonate	

*Many iatrogenic/nosocomial conditions are accompanied by poorly characterized immune defects (e.g., burn injuries and surgery down modulate normal host defense mechanisms), as is diabetes mellitus.
‡HIV and diabetes mellitus predominantly predispose to mucocutaneous candidal infections, and diabetes is also a risk factor for disseminated disease; HIV is a cofactor for, but not an independent risk factor for, disseminated disease.
†Mean time to onset of disease in a recent, large, prospective study was day 22 of hospitalization [13].

factors for disseminated candidiasis are common iatrogenic and/or nosocomial conditions (Table 1). In particular, recent series have shown that 65–90% of patients with disseminated candidiasis had a central venous catheter [19,33,42–45]. As well, in a prospective observational study of ~2,500 cases of candidemia, the mean time to onset of disseminated candidiasis was 22 days of hospitalization [13]. These data emphasize that disseminated candidiasis typically afflicts patients with severe illnesses who have prolonged hospitalizations.

Temporally, host colonization by *Candida* is the first step in the pathogenesis of hematogenously-disseminated candidiasis. This conclusion is derived from the following data: (i) colonization by *Candida* is an independent risk factor for development of disseminated candidiasis [27,46–69]; (ii) patients with higher colonization burdens (i.e., more sites colonized) have a proportionately higher risk of developing hematogenously disseminated disease [50–52]; (iii) colonization temporally precedes disseminated candidiasis [42,53–57]; and (iv) treatments that lower colonization burden simultaneously decrease the risk of fungemia [58]. The key to colonization is adherence of the organism to host surfaces, the importance of which can be inferred from studies demonstrating a correlation between the adhesiveness of different species of *Candida* [59,60] with their relative frequencies of causing invasive infection [12,61–63]. Furthermore, blockade of adhesion diminishes the risk of subsequent infection [64,65].

Use of broad-spectrum antibiotics which suppress the growth of normal bacterial flora increases the burden of *Candida* colonization [66–69] and increases the risk of disseminated candidiasis [2,22,26,70–72]. In one study, the strongest risk factor for nosocomial candidemia was the number of prior antibiotics used, especially when patients who received three to five antibiotics were compared to those receiving two or fewer [73].

Additionally, disruption of normal skin barriers, for example by burn injury [74,75] or percutaneous catheter placement [22,33,40,71–73,76–79], and disruption of gut mucosal barriers by abdominal surgery [18,75,80–82], instrumentation [83,84], induction of mucositis [76,85], or mucosal atrophy from radiation (86) or parenteral nutrition [87], are major risk factors for invasive *Candida* infections. Direct translocation of *Candida* across the gastrointestinal tract of animals [87–89] and humans [90] has been well documented, and gastrointestinal surgery is a well-described clinical risk factor for development of disseminated candidiasis [24,58,88,91–95]. More recently, cardiac surgery has also been described as a major risk for disseminated candidiasis [96–100].

The major form of immunosuppression that predisposes to development of disseminated candidiasis is a defect in innate phagocytic activity. Neutropenia dramatically increases the risk of [4,27,46,47,101,102] and mortality due to disseminated candidiasis [4,47,48,103–105]. Concordant with their well-characterized suppression of phagocyte function [88,106,107], glucocorticoids also increase the risk of disseminated candidiasis [4,105,108]. Similarly, diabetes markedly increases the incidence of both mucocutaneous and disseminated candidiasis [109].

Patients with late stage HIV disease have an extremely high incidence of developing mucocutaneous candidiasis [109,110]. However, HIV infection is not an independent risk factor for disseminated candidiasis. The increased incidence of disseminated candidiasis in patients infected with HIV is attributable to the increased incidence of the usual risk factors for candidemia, including central lines, broad spectrum antibiotics, hospitalization in an intensive care unit, parenteral nutrition, and neutropenia [78,79]. Patients infected with HIV who do not have traditional risk factors for disseminated candidiasis are not at increased risk of developing the disease.

Species distribution

Through the late 1980s, the predominant species causing invasive *Candida* infections was *C. albicans*. Indeed, *C. albicans* adheres most avidly to human tissue *in vitro* [59,60], and in animal models, *C. albicans* is by far the most virulent species of *Candida*

[111–113]. However, since the 1990s there has been a steady increase in the relative frequencies of non-*albicans* species of *Candida* causing disseminated candidiasis. This epidemiological trend has profound consequences for selection of empiric antifungal therapy (see below).

In recent series, *C. albicans* has been responsible for approximately 50% of invasive *Candida* infections, with *C. glabrata* generally the second most common cause of infection in the US and much of Europe, causing 15–25% of cases (Table 2) [12,13,15,18,19,114,115]. In contrast, in Latin America and Spain, *C. parapsilosis* is the second most common cause of invasive candidiasis, with *C. glabrata* the third most common [116–118]. *C. tropicalis* causes 10–20% of cases in most series. The frequency of other species remains low, except in major cancer centers where widespread azole prophylaxis is used. In such centers, *C. krusei* may cause >10% of cases of invasive *Candida* infections [63,119,120].

Multiple studies have investigated risk factors for infection with non-*albicans* species of *Candida*. Numerous studies have reported that exposure to azoles is a risk factor for subsequent development of invasive candidiasis caused by *C. glabrata* or *C. krusei* as opposed to *C. albicans* [121–128]. However, not all studies are concordant. One recent retrospective case control study found no relationship between fluconazole exposure and infection with *C. glabrata* or *C. krusei* compared to *C. albicans* [129], and a second study found that only a quarter of patients infected with *C. glabrata* had been previously exposed to fluconazole [130]. Patients infected with *C. glabrata* have been reported to be older and more debilitated than those infected with *C. albicans* [130,131]. Hence, the cause of the shift away from *C. albicans* is likely multi-factorial.

One reason to suspect that increasing fluconazole use has played a role in the shift towards *C. glabrata* infections is that *C. glabrata* is often resistant to fluconazole due to a drug efflux pump [132]. Overall, the frequency of fluconazole-resistance (MIC ≥16 μg/ml) amongst *C. glabrata* strains has ranged widely from approximately 20–75% [114,133–135]. However the majority of these strains demonstrate 'susceptibility – dose/delivery dependent (SDD)' resistance (MIC 16–32 μg/ml) rather than high level resistance (MIC ≥64 μg/ml) [136], and these isolates may still respond to high doses of fluconazole *in vivo* [136]. Indeed, a recent study found that candidemic patients with higher fluconazole dose/MIC or fluconazole area under the curve (AUC) serum level/MIC ratios had significantly better outcomes than patients with lower dose/MIC or AUC/MIC ratios [137], supporting the concept of SDD resistance. In contrast, virtually all *C. krusei* isolates are intrinsically totally resistant to fluconazole due to an altered target enzyme [138]. Not surprisingly, prior fluconazole use has been shown to increase the likelihood of *C. krusei* infection [27,139].

Table 2 Species breakdown of disseminated candidiasis [12–14,18,19,114]

Species	Percent of cases
C. albicans	≈50%
C. glabrata	≈15–25%
C. parapsilosis	≈10–20%
C. tropicalis	≈15%
*C. krusei**	≈ <3%
Others	≈ <5%

*At cancer centers where significant fluconazole prophylaxis is used, *C. krusei* incidence may cause up to 10–15% of disseminated candidiasis [119,120,134].

Strain acquisition

Although the gastrointestinal tracts of the majority of people are colonized by *Candida*, it is not clear whether the strains that colonize healthy hosts are responsible for causing subsequent invasive disease when those hosts acquire the appropriate risk factors, or whether infections are caused by acquisition of more virulent strains from environmental sources in the nosocomial setting.

Numerous investigations have been undertaken to explore this question, but the results have been mixed. Some studies have found evidence of patient-to-patient spread of single *Candida* isolates [140–143], and one study successfully traced the isolate causing a *Candida* sternal wound infection to a scrub nurse [144]. In contrast, other studies have found that individual colonizing or infecting strains of *Candida* are specific to each patient, suggesting that the source of an infecting strain was indeed endogenous flora that became pathogenic in the compromised hosts [134,145,146]. Similarly, in another study, blood isolates of *Candida* were found to be very similar or identical to strains colonizing patients in 90% of cases of disseminated candidiasis [55]. Overall, these data suggest that in most cases, the source of an infecting strain of *C. albicans* is endogenous flora, but that in certain circumstances transmission of more virulent strains may occur in the nosocomial setting.

Therapeutic strategies

Not only are invasive *Candida* infections extremely common, they are difficult to treat. Even with first-line antifungal therapy, disseminated candidiasis has an

attributable mortality of up to 40% [37,147,148], and a greater than 50% attributable mortality in patients having undergone myeloablative chemotherapy [4,47,149,150]. The mortality of severe sepsis caused by *Candida* is also greater than 50%, and is therefore higher than the mortality from sepsis due to any bacterium, including *Pseudomonas aeruginosa* [151,152]. Unfortunately, newer therapies, such as voriconazole, lipid-based amphotericin formulations, and echinocandins, have not been shown to improve survival of candidemic patients compared to amphotericin B deoxycholate [153–156].

Data are now available supporting the intuitive assumption that delayed initiation of therapy for candidemia is associated with significantly higher mortality, placing a premium on early administration of therapy. Several recent studies have found that mortality from candidemia dramatically increased if active antifungal therapy was initiated more than 24 hours after positive blood cultures were drawn [157,158]. Indeed, initiation within 12 h of drawing positive blood cultures appeared to be necessary to maximize outcomes [158]. In a separate study of critically ill patients with severe sepsis, each hour of delay in initiating active antifungal therapy after the onset of hypotension increased mortality from candidemia by $\sim$5% [159].

Because of the difficulties in confirming the diagnosis with laboratory studies, empiric administration of therapy often must be based on a clinical diagnosis of disseminated candidiasis. Therefore, a high index of suspicion must be maintained in the appropriate patient population to enable clinical diagnosis and early treatment. A clinical diagnosis of disseminated candidiasis is typically made in a patient with signs, symptoms, and laboratory features consistent with infection, who does not respond to broad-spectrum antibacterials, and who has risk factors for disseminated candidiasis. In such patients, early empiric therapy is appropriately administered. If a clinical response is seen (e.g., decreasing white blood cell count, defervescence, improving hemodynamics, etc.), a clinical diagnosis of disseminated candidiasis can be made retrospectively.

Consensus guidelines on the empiric treatment of disseminated candidiasis are available [136,160]. In general, due to its favorable toxicity profile, high oral bioavailability, low cost, and impressive efficacy in randomized clinical trials, fluconazole therapy is preferred in hemodynamically stable patients. Recently, the results of a clinical trial presented at an international meeting have suggested that fluconazole may be inferior in efficacy compared to anidulafungin for invasive candidiasis [161]. However, to date the study is not available in a peer-reviewed publication. Furthermore, caspofungin and micafungin, which are similar to anidulafungin (see below), have been shown to be non-inferior to amphotericin B deoxycholate [156] and liposomal amphotericin B [162], respectively, as well as to each other [163]. Since two large, randomized trials definitively concluded that fluconazole was equivalent in efficacy to amphotericin B deoxycholate for candidemia [164,165], and polyenes have been shown to be equivalent in efficacy to echinocandins, the mathematical transitive principle suggests that all three classes of antifungals are likely equivalently efficacious for the treatment of disseminated candidiasis (i.e., if azole = polyene, and polyene = echinocandin, then azole = echinocandin; Table 3, Table 5). For now, it is reasonable that fluconazole remain a 'work-horse' antifungal for disseminated candidiasis in stable, non-neutropenic patients [166].

Because fluconazole may not adequately treat a significant component of *C. glabrata* isolates, a broader spectrum agent, such as a polyene, echinocandin, or possibly voriconazole, is preferred when there is an urgency to treat for all possible species, such as in unstable patients. Certainly fluconazole should be avoided when an azole-resistant strain is likely to be causing the infection, such as in a patient who is known to be colonized with *C. glabrata* or *C. krusei*, or in a patient exposed to fluconazole within the past 30 days. Neutropenic patients represent an additional subset of patients in which it may be advisable to avoid static azole therapy. The use of a static agent such as

Table 3 Summary of randomized clinical trails for treatment of invasive candidiasis

Author	Conclusion based on primary endpoint
Rex *et al*. [165]	Fluconazole equivalent to amphotericin B deoxycholate
Phillips *et al*. [164]	Fluconazole equivalent to amphotericin B deoxycholate
Rex *et al*. [168]	Fluconazole plus amphotericin B deoxycholate equivalent (trend to superior) vs. fluconazole plus placebo
Kullberg *et al*. [153]	Voriconazole equivalent to amphotericin B deoxycholate followed by fluconazole
Mora Duarte *et al*. [156]	Caspofungin equivalent to amphotericin B deoxycholate
Ruhnke *et al*. [162]	Micafungin equivalent to liposomal amphotericin B
Reboli *et al*. [161]	Anidulafungin superior to fluconazole
Betts *et al*. [163]	Micafungin 100 mg/d equivalent to micafungin 150 mg/d equivalent to caspofungin 70 mg $\times$1, then 50 mg/d

fluconazole in candidemic neutropenic patients should be considered carefully, especially in neutropenic patients with sepsis. However, the practice of using fluconazole in stable neutropenic patients is becoming more popular, in general [136].

If a broader spectrum agent is required, amphotericin B deoxycholate, amphotericin B lipid complex, liposomal amphotericin B, voriconazole, caspofungin, micafungin, or anidulafungin are all acceptable first-line agents. The primary distinction between these agents relates to preferred species coverage, and differences in adverse effects. Unfortunately, the polyenes are becoming less effective against the two azole-resistant species, *C. glabrata* and *C. krusei*. Guidelines therefore recommend using higher than normal doses of polyenes against these species (i.e., 0.7–1 mg/kg/d for amphotericin B deoxycholate, or 5–10 mg/kg for lipid amphotericins [136,166]). Because higher doses of polyenes increase the risk of nephrotoxicity, and voriconazole may be ineffective for certain strains of *C. glabrata*, there is an emerging consensus that echinocandins may be the drugs of choice for *C. glabrata* and *C. krusei* infection [166].

Based on data from four large, randomized, comparative studies, all three echinocandins (caspofungin, micafungin, anidulafungin) are reasonable first line options for invasive *Candida* infections (Tables 3, 4, 5) [156,161–163]. The three echinocandins are structurally, pharmacokinetically, and pharmacodynamically similar, all three have similar activities in animal models, and all three have similar randomized clinical trial data (Table 4). Hence, there is currently no obvious preference for one echinocandin over another for the treatment of invasive *Candida* infections. These drugs are clearly preferred in the setting of renal failure, where polyenes cannot be used. In contrast, polyenes or azoles may be preferred for treatment of *C. parapsilosis*, which tends to have higher minimum inhibitory concentrations (MICs) against the echinocandins. However, it must be emphasized that echinocandin MICs have not been shown to correlate with clinical outcomes from invasive candidiasis [167]. Furthermore, in their pivotal, phase III trials, caspofungin or micafungin were found to be as effective against *C. parapsilosis* as amphotericin B deoxycholate or liposomal amphotericin B, respectively [156,162]. In contrast, in its pivotal study, anidulafungin appeared to be less effective than fluconazole at mediating microbiological eradication of *C. parapsilosis* invasive infection [161]. The clinical significance of this finding is not clear.

Table 4 The echinocandins: a study in similarity

	Anidulafungin	Caspofungin	Micafungin
Pharmacology			
Daily Dose*	100 mg	50 mg	100 mg
Half-life ($t_{½}$) [329]	>24 h	9–11 h	11–15 h
Cmax [330–332]	8.6 μg/ml	12 μg/ml	7 μg/ml
Trough [330–332]	3 μg/ml	1.4 μg/ml	3 μg/ml
AUC_{24} [329]	110	98	115
P_{450} Interactions	Minimal	Yes	Minimal
Dose adjustments			
Renal	No	No	No
Hepatic	No	½ dose mod	No (mild-mod)
Transplant Meds	No	Yes	No
Randomized clinical trials for invasive candidiasis			
	Anidulafungin [161]	Caspofungin [156]	Micafungin [162]
Double-blind?	Yes	Yes	Yes
Mostly candidemia? Yes	Yes	Yes	Yes
Number of patients	261	239	531
Comparator arm	Fluconazole	AmB	LAmB
Neutropenic Patients?	Minimal	Minimal	Significant (>75)
Non-inferior?	Yes–?superior	Yes	Yes
FDA indication for candidemia?	Yes	Yes	No
Trial published?	No	Yes	No

AmB = amphotericin B deoxycholate; LAmB = liposomal amphotericin B.
*Daily Dose for invasive candidiasis; anidulafungin with 200 mg ×1 load then 100 mg qd, caspofungin with 70 mg ×1 load, then 50 mg qd; no load for micafungin.

Table 5 Summary of antifungal treatments for nosocomial invasive fungal infections

Disease	First line antifungal(s)	Alternative strategies
Candida	• Fluconazole 400–800 mg qd • Voriconazole 200 mg bid • Amphotericin B deoxycholate 0.7 mg/kg/d (0.7–1 mg/kg/d for *C. glabrata* or *C. krusei*) • Liposomal amphotericin B 3–5 mg/kg/d • Amphotericin B lipid complex 5 mg/kg/d • Caspofungin 70 mg ×1, then 50 mg qd • Micafungin 100 mg qd • Anidulafungin 200 mg ×1, then 100 mg qd	
Aspergillus	• Voriconazole 200 mg bid (300 mg bid for CNS disease)	Combination voriconazole + (echinocandin or polyene)
Mucor	• Amphotericin B deoxycholate 1–1.5 mg/kg/d • Liposomal amphotericin b 5–10 mg/kg/d (for CNS disease 10–15 mg/kg/d)* • Amphotericin B lipid complex 5–10 mg/kg/d	Combination polyene + echinocandin (based on mouse data [290]), posaconazole, deferasirox iron chelation
Fusarium	• Voriconazole 200 mg bid	Voriconazole + polyene
Other molds	• Voriconazole 200 mg bid (add terbinafine 250 mg bid for *S. prolificans*) • Polyenes depending on isolate's susceptibility	Voriconazole + polyene

*Liposomal amphotericin B may be preferred (see text).

Combination therapy

For many years, there were concerns about combining azoles with polyenes for the treatment of fungal infections. Since azoles inhibit production of ergosterol, which is the primary target of polyenes, these drug classes were theorized to be antagonistic. A recent, large, randomized clinical trial has definitively answered this nagging question. Rex *et al*. randomized non-neutropenic patients with candidemia to receive fluconazole plus placebo or fluconazole plus amphotericin B deoxycholate [168]. The combination arm trended to superiority in time to failure analysis ($P = 0.08$), and was superior in secondary analysis of the proportion of clinical success and in microbiological clearance of bloodstream infection. Therefore, combination therapy was clearly not antagonistic, and showed evidence of additive benefit.

This proof-of-principle study aside, the question remains, what is the role of combination antifungal therapy for the treatment of disseminated candidiasis in real-world, clinical settings? Rex *et al*. found that the entire benefit of combination azole plus polyene therapy was restricted to patients with intermediate APACHE II scores [168]. Patients with low APACHE II scores responded well to monotherapy, and patients with very high APACHE II scores did not respond well to either mono- or combination therapy. Thus there does not appear to be any role for combination therapy for hemodynamically stable patients with disseminated candidiasis. On the other hand, it would be rather nihilistic to refuse to use more aggressive combination therapy because a patient was too sick. Another argument against combination therapy is the increased cost compared to monotherapy. However, this argument is mitigated by the low drug acquisition cost of amphotericin B deoxycholate and fluconazole, the combination of which would be far cheaper to administer than monotherapy with voriconazole, lipid amphotericin, or any of the echinocandins.

Given that a major benefit of combination therapy was more reliable microbiological eradication, combination azole plus polyene therapy may be reasonable to consider in select patients with high burdens of organism who are seriously ill from disseminated candidiasis. However, combination therapy cannot be recommended for routine care of disseminated candidiasis based on the available data, and there are no data in humans for antifungal combinations other than polyenes plus azoles.

Aspergillus

Frequency

Like *Candida*, *Aspergillus* has been recognized for decades as a source of invasive disease in a variety of immunocompromising conditions. The severity of

immunocompromise required to predispose to invasive *Aspergillus* infections is greater than for *Candida* infections, as evidenced by the fact that: (i) in contrast to *Candida, Aspergillus* almost never causes invasive disease in immunocompetent hosts with typical nosocomial risk factors (i.e., central venous catheters, post-surgery, on antibiotics, on parenteral nutrition, etc.); (ii) *Aspergillus* infections predominantly occur in patients with intensive immunocompromising conditions (i.e., hematologic malignancies, organ or stem cell transplant recipients, or prolonged, high dose steroids) [169]; (iii) *Aspergillus* infections predominantly occur after a longer average duration of neutropenia than *Candida* infections [169]; and (iv) *Aspergillus* infections predominantly occur after a longer average time post solid-organ or hematopoietic stem cell transplantation than *Candida* infection [169].

Overall, *Aspergillus* is the second most common cause of nosocomial, invasive fungal infections, with an incidence of approximately 5 per 100,000 population in the US [1]. Thus there are approximately four cases of invasive *Candida* infections for every case of invasive aspergillosis. However, outcomes from invasive aspergillosis are even worse than for disseminated candidiasis. Various studies have demonstrated crude mortality rates of 45% to >80% [170–174]. The primary predictor of survival is time to reversal of the underlying immune defect (e.g., neutropenia). Hence, mortality rates in hematopoietic stem cell transplant (HSCT) recipients have been reported to be as high as 95% [175].

Habitat

Aspergillus spp. are ubiquitous molds whose habitat includes soil, fresh fruits, and vegetables. *Aspergillus* infection is acquired primarily by inhaling spores [176,177]. The environmental sources of inhaled *Aspergillus* conidia have been extensively investigated. Well-documented sources include dust and water droplets. Specifically, hospital water systems have been implicated as potential sources of *Aspergillus* spores that cause disease in patients [178]. Increased spore density in the air resulting in outbreaks of invasive aspergillosis has been repeatedly documented to occur during times of construction [179–183], although some studies have not concurred [184,185]. In their study, Loo *et al*. [179] found that the incidence of invasive aspergillosis was 3.18/1000 days before hospital construction began, and rose to 9.88/1000 days during construction. Specific interventions (installation of wall-mounted portable high-efficiency particulate air (HEPA)-filter air purifiers, special paint, new non-perforated ceiling tiles, window sealing, replacement of horizontal blinds, and improved cleaning measures) resulted in a reduction of the infection rate to 2.91/1000 days.

Epidemiologic studies similarly demonstrate that the incidence of invasive aspergillosis in the at-risk population can be reduced remarkably by reducing the *Aspergillus* spore count in the environment. This has been achieved by insisting that patients wear masks when outside of their rooms [186], and by introducing HEPA filtration systems [187,188] and laminar airflow systems in patient quarters [189,190]. However, more recently the precise utility of HEPA-filtration at improving survival in high-risk cancer patients has been a controversial issue [191,192].

Species distribution

As with treatment of candidal infections, the species of *Aspergillus* responsible for infection can impact therapeutic decisions. *A. fumigatus* is the dominant species causing invasive infection [193], with *A. flavus* and *A. niger* being less common causes. Fortunately, these species are susceptible to polyenes. In contrast, *A. terreus* is a particular problem because it is typically resistant to polyenes [194], and clinical failures with amphotericin B deoxycholate are well described [195]. Fortunately, *A. terreus* is often susceptible to voriconazole [194]. A retrospective study of proven or probable *A. terreus* infections found that voriconazole resulted in decreased mortality (55.8 vs. 73.4%) at 12 weeks compared to other antifungals, including lipid or non-lipid polyenes [172]. In another retrospective study of patients with invasive aspergillosis between 1995 and 2001, *A. terreus* was second only to *A. fumigatus* in incidence and had a worse response to antifungal therapy (39% vs. 28%) [196].

Risk factors

The biggest risk factors for invasive aspergillosis are hematologic malignancy, HSCT (especially allogeneic), solid-organ transplant (heart-lung greatest, kidney least), corticosteroid administration, and advanced HIV disease (largely end-stage Acquired Immunodeficiency Syndrome (AIDS) in the era prior to highly active antiretroviral therapy (HAART)) [170,171]. The site of disease tends to correlate with the underlying condition. For example, invasive pulmonary aspergillosis occurs more often in lung and heart-lung transplants than in other populations [197]. In a review of 342 patients with AIDS, invasive pulmonary aspergillosis tended to occur when CD4 count was below 50 cells/μl, and was associated with steroid use,

neutropenia, and other opportunistic infections [198,199].

As mentioned, recipients of HSCT, and especially allogeneic HSCT, are at particularly high risk for invasive aspergillosis. In such patients, the incidence of invasive *Aspergillus* infections is bimodal, peaking at approximately 2 weeks and again at 3 months post-transplantation [200–202]. Invasive aspergillosis occurring during the earlier peak is due to prolonged neutropenia in the immediate post-transplant period. In contrast, invasive aspergillosis occurring during the second peak period is typically due to corticosteroid therapy for graft-versus-host disease (GVHD) [8,202]. For example, Marr *et al*. found that the probability of developing invasive aspergillosis was 5% at 2 months, 9% at 6 months, and 10% at 12 months after HSCT [202]. The probability increased slightly thereafter to 11.4% at 5 years. Risk factors included higher age, comorbidities, and type of transplant, with cord blood recipients and HLA-mismatched donors more susceptible. Late incidence was associated with neutropenia, acute or chronic GVHD, and CMV or respiratory viral infections [202].

Diagnosis

As for disseminated candidiasis, making the diagnosis of invasive aspergillosis can be difficult. In contrast to *Fusarium* (see below), *Aspergillus* rarely grows from blood, CSF, or other sterile sites [203]. Furthermore, because *Aspergillus* is ubiquitous in the environment, finding the organism in non-sterile material, such as BAL fluid, skin, etc., does not definitely establish disease [204]. Nevertheless, the presence of the organism in any material taken from a highly immunocompromised host is extremely concerning. For example, Yu *et al*. reported that 17 of 17 patients with leukemia and/or neutropenia with *Aspergillus* in respiratory secretions had invasive pulmonary aspergillosis, 16 of whom died [205]. In contrast, none of the immunocompetent patients with *Aspergillus* in their sputum had invasive disease. Unfortunately, in another series of 23 consecutive patients with histologically proven invasive aspergillosis, only 30% had positive bronchoscopic cultures or cytology. Hence, failure to recover *Aspergillus* from respiratory secretions does not allow aspergillosis to be ruled out, whereas recovery of the mold provides compelling evidence in support of the diagnosis.

One difficulty in diagnosing invasive aspergillosis is defining the precise criteria required to establish the diagnosis. Fortunately, consensus European Organization for the Research and Treatment of Cancer (EORTC)/Mycoses Study Group (MSG) diagnostic criteria are now published [206]. The gold standard for diagnosis remains identification of the organism by histopathology and/or growth in culture from tissue biopsy or aspirate from a sterile site. However, culture of the organism from non-sterile sites (such as sputum or bronchoalveolar lavage) from an immunocompromised host who has clinical evidence of infection can be utilized to support a probable diagnosis of invasive aspergillosis [206].

In histopathological specimens, *Aspergillus* is best seen by Gomori methenamine silver or Periodic acid Schiff stains, but the hyphae can be difficult to distinguish from other invasive molds [207]. More recently, the galactomannan ELISA has been utilized as a diagnostic tool for invasive aspergillosis. The literature on this assay is highly variable. Mennink-Kersten *et al*. have admirably reviewed the wide variety of potential causes of the variable sensitivity reported over the years for the galactomannan assay [208]. These authors thoroughly discuss the microbiological, epidemiological, and host variables that can affect the reliability of the galactomannan assay, underscoring that the precise reasons for the variable test performance are poorly understood. Overall, a recent meta-analysis found that the sensitivity of the test was 0.73 (95% CI, 0.61–1.0) and specificity was 0.81 (95% CI, 0.76–0.87) for proven invasive aspergillosis [209]. For proven or probable invasive aspergillosis, the sensitivity was 0.69 (95% CI, 0.59–0.79) and specificity was 0.89 (95% CI, 0.84–0.94), making the test possibly useful at ruling out disease in low pre-test probability populations, but not useful at ruling in the disease [209]. In general, studies from Europe have reported more favorable test characteristics than studies from the US, for unclear reasons. Furthermore, a variety of conditions have been reported to result in false positive assays, including a lowered threshold for test positivity [209], *Bifidobacterium* lipoglycan [210,211], and use of concurrent β-lactam antibiotics, particularly piperacillin-tazobactam [212–214]. The heterogeneity between the various published studies on this topic makes it difficult to extrapolate these published datasets to individual health care settings [209].

The serum β glucan assay is also now available for the diagnosis of invasive mycoses, including aspergillosis, but the published experience with the assay is limited and of mixed results [215,216]. Therefore the precise utility of the β glucan assay in diagnosing invasive aspergillosis remains unclear. More recently, PCR has been evaluated as a potential diagnostic modality. For example, PCR was analyzed prospectively in 84 stem-cell transplant patients, and was found

to have a sensitivity of 100% for invasive aspergillosis, preceding the development of symptoms by a median of 2 days and clinical diagnosis by 9 days. No patient with a negative PCR developed invasive aspergillosis [217]. However, the PCR study has not yet been validated sufficiently and is not licensed as a diagnostic modality for aspergillosis.

The utility of radiologic studies in diagnosing invasive pulmonary aspergillosis has been extensively studied, mostly using computerized tomography (CT) scanning. Emphasis on finding radiologic clues stems from the low sensitivity of other diagnostic methods. The most common radiographic manifestations of invasive pulmonary aspergillosis on CT scans are nodules or patchy consolidations [218,219]. The classic 'halo' sign, an attenuated area around a nodule, has traditionally been associated with aspergillosis but is not necessarily specific and can be seen in other infectious and non-infectious conditions [220]. However, in the setting of immunocompromise, this sign becomes more specific for aspergillosis. For example, in a population of neutropenic and other immunocompromised patients, Horger *et al*. found the 'halo' sign to have a sensitivity of only 30.2% but a specificity of 100% for aspergillosis [221]. However, the sensitivity of the 'halo' sign is dependent upon timing of the study relative to the diagnosis of invasive aspergillosis. For example, the 'halo' sign has been shown to be highly sensitive early on, with 80–90% of chest CT scans showing a 'halo' sign on the day of diagnosis of invasive pulmonary aspergillosis [218,219]. Other studies have also reported a sensitivity of 95–100% for the 'halo' sign in the setting of neutropenic fever unresponsive to antibacterial agents [222,223]. In studies of serial CT scans, the sensitivity of the 'halo' sign dramatically waned over time, from 88–96% on the day invasive aspergillosis was diagnosed to 17–19% by approximately two weeks later [219,224].

In summary, the CT scan has become an invaluable resource for early detection of pulmonary aspergillosis in immunocompromised patients. Many experts advocate checking high resolution chest CTs in all at-risk patients who are febrile and not-responding to antibacterials, regardless of chest X-ray findings or lack of pulmonary symptoms.

Treatment

Amphotericin B deoxycholate was the mainstay of therapy for invasive aspergillosis for half a century, until Herbrecht *et al*. reported their landmark study comparing the efficacy of voriconazole with amphotericin B deoxycholate for patients with invasive aspergillosis [225]. Nearly 400 patients with invasive aspergillosis by standard definitions were randomized to receive voriconazole or amphotericin B deoxycholate, with or without other licensed antifungal therapy. The majority of patients in the modified intention-to-treat analysis had leukemia or other hematologic malignancies, and just under half were neutropenic. The only difference between the groups was that more cases of definite invasive aspergillosis received voriconazole. Voriconazole was superior to standard antifungal therapy by both the primary endpoint (global response at 12 weeks), and multiple secondary endpoints, including survival at 12 weeks (70.8 vs. 57.9%, $P = 0.02$). In addition, fewer adverse events were noted in the voriconazole group. As a result of this study, voriconazole is now considered the gold-standard first-line therapy for invasive aspergillosis (Table 5).

Nevertheless, while voriconazole was clearly superior to standard antifungal therapy, the overall response rate was only 49.7%, and the rate of complete response was only 20.8% [225]. These rates of success are hardly indicative of highly effective antimicrobial therapy, which has prompted continued debate about the potential for combination therapy to improve outcomes (see below).

Other antifungals with activity against *Aspergillus* include lipid formulations of amphotericin, as well as itraconazole and the echinocandins. Amphotericin B colloidal dispersion (ABCD), which is rarely used due to its increased infusional toxicity, was compared with amphotericin B deoxycholate in a randomized, double-blinded study of patients with invasive aspergillosis (226). While ABCD resulted in diminished nephrotoxicity, it caused increased infusional toxicity and was not superior to amphotericin B deoxycholate in any clinical endpoint, including response rate, mortality, and mortality due to fungal infection [226].

Liposomal amphotericin B, amphotericin B lipid complex, and the echinocandins, caspofungin and micafungin, have been studied in salvage settings for patients with invasive aspergillosis refractory to, or intolerant of, first-line therapy. Response rates to each of these drugs were similar in these retrospective salvage studies, with complete and overall response rates ranging from 22 to 63% and 37–67% [171,227–231].

Itraconazole has been extensively studied in the treatment of invasive aspergillosis but not in randomized comparative trials [232–235]. In general, itraconazole has compared favorably with amphotericin B except in central nervous system disease where itraconazole has inferior penetration. It has also been shown

to be of benefit in patients who experienced disease relapse or failure of prophylaxis on amphotericin B.

Posaconazole is the most recently introduced azole. Posaconazole has significant *in vitro* and animal model activity against invasive aspergillosis [236]. Posaconazole has been administered as salvage therapy to patients with refractory invasive aspergillosis, although the number of cases is too small to allow for any significant conclusions to be drawn regarding its efficacy [236,237].

In general, each of these therapies is a reasonable consideration in a salvage setting or as part of a combination regimen, but data are not available to indicate that they should be considered first-line therapies on par with voriconazole.

Combination therapy

Whether or not to use combination antifungal therapy for invasive aspergillosis is one of the most controversial issues in all of Infectious Diseases. Numerous symposia at international meetings have been and continue to be dedicated to this topic, and dozens of studies have been published comparing monotherapy versus combination therapy *in vitro* and in animal models [238]. However, the heterogeneity of results in these studies, combined with the dearth of prospective data in humans, has led to a lack of consensus on this issue in the mycology community.

Recently, Marr et al reported the results of their retrospective evaluation of 47 patients with proven or probable aspergillosis who had failed standard therapy with a polyene and who were then treated with either voriconazole alone or combined with caspofungin [239]. Most of the patients were HSCT recipients and had proven pulmonary aspergillosis. The investigators found that combination voriconazole plus caspofungin therapy was associated with an improved three month survival compared to voriconazole alone (hazard ratio of death 0.42, $P=0.048$). By multivariate analysis, combination therapy was independently associated with improved survival compared to voriconazole alone (hazard ratio $=0.28$, $P=0.011$).

Similarly Singh *et al*. prospectively studied the combination of caspofungin and voriconazole as primary therapy for invasive aspergillosis in solid-organ transplant recipients [240]. This cohort was then retrospectively compared to a control group, most of whom had received a lipid formulation of amphotericin B for invasive aspergillosis. The result was a non-significant trend to benefit of combination therapy over monotherapy in 90-day survival (67.5 vs. 51%, $P=0.117$). However, the study was not adequately powered to detect a difference of such magnitude. Further analysis revealed improved survival in the combination caspofungin plus voriconazole group in those with confirmed *A. fumigatus* infection ($P=0.019$) or renal failure ($P=0.022$). While certainly not conclusive, the data from Marr *et al*. [239] and Singh *et al*. [240] suggest that combination therapy may well be of benefit in immunocompromised patients with invasive aspergillosis.

In light of the lack of prospective, randomized data in humans on this issue, perhaps it is more relevant to briefly consider the merits and deficits of combination therapy from a theoretical perspective. The most compelling arguments against the use of combination antifungal therapy are: (i) there are no data to prove combination therapy is more effective; (ii) combination therapy adds cost; and (iii) combination therapy may add toxicity. Each of these points is true, but each is mitigated by opposing arguments. For example, lack of proof of superiority of combination therapy is not the same as proof of lack of superiority of combination therapy, especially considering the lack of randomized clinical studies evaluating this question. Furthermore, high risk patients (such as transplant patients) likely incur total hospital costs that are already extremely high, representing a significant investment of health care dollars. The additional cost of a second antifungal agent likely represents a small fraction of their overall costs of care. As well, echinocandins have very favorable adverse event profiles, and are unlikely to add significant toxicity to an azole-based regimen.

The most compelling argument in favor of combination therapy is the abysmal success rate of monotherapy for invasive aspergillosis. To reiterate, in its pivotal phase III trial, voriconazole was clearly superior to standard antifungal therapy, but still resulted in only a $\approx$20% complete response rate [225]. That antifungal studies for mold infections report success as a composite of complete and partial responses is a telling fact. There are very few realms in Infectious Diseases where partial responses are considered acceptable, or where 20% complete response rates are considered acceptable.

In the end, an individual practitioner's belief regarding the merits and deficits of combination therapy boil down to perspective. Is it preferable to treat with maximal aggressiveness until such time as combination therapy is proven not to be of benefit, or is it preferable to treat with less aggressive monotherapy until such time as more aggressive combination therapy is proven to be of benefit? In light of the very poor outcomes associated with monotherapy, we believe that combination therapy should be strongly considered for patients with invasive aspergillosis until such time as combina-

tion therapy is proven to be no better than monotherapy (Table 5).

Mucormycosis

Frequency

Mucormycosis is a fungal emergency that virtually always occurs in patients with defects in host defense and/or with increased available serum iron [241]. Mucormuycosis is less common than other opportunistic fungal infections, such as those caused by *Candida* and *Aspergillus* spp. One population-based study estimated the incidence of mucormycosis to be 1.7 cases per million people per year, which translates to approximately 500 cases per year in the United States (6). In autopsy series, the prevalence of mucormycosis has ranged from 1 to 5 cases per 10,000 autopsies, making the infection 10–50 fold less common than invasive *Candida* or *Aspergillus* infections [242–244].

In recent years, the epidemiology of mucormycosis has shown an alarming trend. Mucormycosis, formerly virtually always community-acquired and often in the setting of diabetic ketoacidosis, has rapidly become a nosocomial infection in patients with malignancy or undergoing organ transplantation or HSCT [245]. Indeed, in patients undergoing allogeneic bone marrow transplantation, the prevalence of mucormycosis has been described to be as high as 2–3% [246,247]. Iatrogenic outbreaks have also been described to occur in the setting of contaminated wound dressings or medical instruments (see below).

Species distribution

Fungi belonging to the order Mucorales are distributed into 6 families, all of which can cause cutaneous and deep infections [248]. Species belonging to the family Mucoraceae are isolated more frequently from patients with mucormycosis than any other family. Among the Mucoraceae, *Rhizopus oryzae* (*Rhizopus arrhizus*) is by far the most common cause of infection [248,249]. Other less frequently isolated species of the Mucoraceae family that cause a similar spectrum of infections include *Rhizopus microsporus* var. *rhizopodiformis, Absidia corymbifera, Apophysomyces elegans, Mucor* species, and *Rhizomucor pusillus* [248–251]. Increasing cases of mucormycosis have been also reported due to infection with *Cunninghamella* spp. (in Cunninghamellaceae family) [249,252–255]. To date, rare case reports have demonstrated the ability of species belonging to the remaining four families to cause mucormycosis [248,256–259].

Risk factors and disease manifestations

Nosocomial mucormycosis has been associated with iatrogenic immunosuppression [245,260,261], or a variety of procedures or devices used in hospitals, including antifungal prophylaxis [260,262], contaminated bandages or medication patches [263,264], intravenous catheters [265–268], and even tongue depressors (see below) [269–271]. At transplant centers there has also been a rise in the incidence of mucormycosis [262,272]. For example, at the Fred Hutchinson Cancer Center, Marr *et al*. have described a doubling in the number of cases from 1985–1989 to 1995–1999 [9]. Similarly, Kontoyianis *et al*. have described a greater than doubling in the incidence of mucormycosis in transplant patients over a similar time-span [261]. In patients undergoing hematological stem cell transplantation, mucormycosis develops at least as commonly in non-neutropenic periods as in neutropenic periods. For example, two major transplant centers have recently reported that more than half the cases of mucormycosis occurred more than 90 days after transplantation [9,246].

Major risk factors for mucormycosis in the transplant setting include underlying myelodysplastic syndrome (possibly due to iron overload from repeated blood transfusions), and GVHD treated with steroids [9,246,272,273]. Administration of anti-thymocyte globulin may also be a risk for mucormycosis [272]. Although less than half of these patients are neutropenic at the time of disease onset, prolonged neutropenia is a risk factor for mucormycosis in this setting [260], as are diabetes mellitus and steroid use [260].

The role of antifungal prophylaxis in predisposing patients to developing mucormycosis is increasingly being described. Prophylaxis with either itraconazole [260] or voriconazole [247,262,274–276] have been implicated in predisposing to mucormycosis, and these cases have typically presented with disseminated mucormycosis, the most lethal form of disease.

Mucormycosis of the lung occurs most commonly in leukemic patients who are receiving chemotherapy, or in patients undergoing HSCT. Indeed, the pulmonary form of the disease is the most common form found in neutropenic or stem cell transplant patients [9,277]. In contrast, soft tissue infections occur in patients with disrupted cutaneous barriers, either as a result of traumatic implantation of soil, maceration of skin by a moist surface [264,278], or in nosocomial settings via direct access through intravenous catheters or subcutaneous injections [265,279,280]. Contaminated surgical dressings have also been implicated as a source of

cutaneous mucormycosis [263,281]. Cutaneous mucormycosis has also occurred in the context of contaminated tape used to secure an endotracheal tube in a ventilated patient [278].

Recently an iatrogenic outbreak of gastric mucormycosis occurred due to contamination of the wooden applicators used to mix drugs that were poured down the patients' nasogastric feeding tubes [270]. These patients presented with massive gastric bleeds. The diagnosis was made by culture of gastric aspirates and culture of the box of wooden tongue depressors.

Diagnosis

There are no reliable serologic, PCR-based, or skin tests for mucormycosis. Therefore, the diagnosis should be made by biopsy of infected tissues. The biopsy should demonstrate the characteristic wide, ribbon-like, aseptate, hyphal elements that branch at right angles. The organisms are often surrounded by extensive necrotic debris. Other fungi, including *Aspergillus, Fusarium,* or *Scedosporium* may look similar to the mucorales on biopsy. However, these molds have septae, are usually thinner, and branch at acute angles. The genus and species of the infecting organism can only be determined by culture of the infected tissue. However, the organism is rarely isolated from cultures of blood, CSF, sputum, urine, feces or swabs of infected areas.

A concept that is frequently poorly grasped by clinicians inexperienced with mucormycosis is that the initial imaging study is frequently negative or has subtle findings [241]. Radiographic findings lag clinical progression in this disease, and a negative imaging study does not provide a rationale to delay more aggressive diagnostic maneuvers (e.g., sinus endoscopy or bronchoscopy with biopsy) if clinical suspicion is high.

Disease manifestations of invasive aspergillosis and mucormycosis may be similar, and both diseases affect similar populations of high-risk cancer or transplant patients. However, it is critical to determine if antifungal coverage for mucormycosis must be included, since therapy for mucormycosis tends to be active against aspergillosis, but therapy for aspergillosis is not necessarily active against mucormycosis (discussed below). In this regard, Chamilos *et al.* performed a retrospective comparison of cancer patients who developed pulmonary mucormycosis or pulmonary invasive aspergillosis to determine if clinical or radiographic findings could distinguish the two diseases [282]. By logistic regression analysis, cancer patients with concomitant invasive sinusitis were 25-fold more likely to have pulmonary mucormycosis than aspergillosis, and patients receiving voriconazole prophylaxis were almost 8-fold more likely to have mucormycosis. On the initial pulmonary CT scan, the presence of multiple nodules or pleural effusion imparted a 20-fold or 5-fold increased risk of mucormycosis compared to aspergillosis, respectively. No other clinical or radiographic findings could distinguish the two diseases.

Treatment

Four factors are critical for eradicating mucormycosis: rapidity of diagnosis, reversal of the underlying predisposing factors (if possible), appropriate surgical debridement of infected tissue, and appropriate antifungal therapy. Early diagnosis is important because small, focal lesions can often be surgically excised before they progress to involve critical structures or disseminate [283]. Unfortunately, there are no serologic or PCR-based tests to allow rapid diagnosis. As mentioned, autopsy series have reported that up to half the cases of mucormycosis are diagnosed post-mortem [243,254,284], underscoring the critical need to maintain a high index of clinical suspicion and to aggressively pursue diagnostic biopsy. Correcting or controlling predisposing problems is also essential for improving the treatment outcome. Specifically, it is critical to maintain tight control of diabetes and to immediately resolve any acidosis. Discontinuation or dose reduction of corticosteroids should be strongly considered when the diagnosis of mucormycosis is made.

Until recently, only members of the polyene class, including amphotericin B deoxycholate or its lipid-derivatives, had been demonstrated to have activity against the agents of mucormycosis. Because the various species that cause mucormycosis have a broad range of susceptibility to amphotericin, the recommended dose of amphotericin B deoxycholate has been 1–1.5 mg/kg/d [250,251,285], which results in a very high toxicity rate. Fortunately, new therapies have become available that have the potential to impact outcomes of mucormycosis.

The lipid formulations of amphotericin are significantly less nephrotoxic than amphotericin B deoxycholate and can be safely administered at higher doses for a longer period of time. Several case reports and case series of patients with mucormycosis have documented successful outcomes with either liposomal amphotericin B or amphotericin B lipid complex [155,286–288]. Although there are no head to head clinical studies comparing the efficacy of liposomal amphotericin B to amphotericin B lipid complex for mucormycosis, more data are available supporting the use of liposomal

amphotericin B than amphotericin B lipid complex. For example, in a murine model of disseminated *R. oryzae* infection in mice in diabetic ketoacidosis, high dose liposomal amphotericin B (15 mg/kg/d) was considerably more effective than amphotericin B deoxycholate (1 mg/kg/d), nearly doubling the survival rate [289]. In contrast, amphotericin B lipid complex (5, 20, or 30 mg/kg/d) did not improve survival compared to placebo or amphotericin B deoxycholate in our murine model of disseminated *R. oryzae* infection [290,291]. Furthermore, relevant to the treatment of central nervous system mucormycosis, a rabbit study demonstrated that liposomal amphotericin B penetrated brain parenchyma at levels more than 5-fold above those of amphotericin B lipid complex [292]. In fact, the brain levels of amphotericin B lipid complex were less than or equal to the levels of amphotericin B deoxycholate, despite the fact that amphotericin B lipid complex was administered at a 5-fold higher dose. These animal studies are complemented by a recent retrospective review of 120 cases of mucormycosis in patients with hematological malignancies, which demonstrated that treatment with liposomal amphotericin was associated with a 67% survival rate, compared to 39% survival when patients were treated with amphotericin B deoxycholate ($P=0.02$, χ^2) [293]. No comparable dataset has been published reviewing the effect of amphotericin B lipid complex in this setting.

Until direct comparisons of the efficacy of liposomal amphotericin B versus amphotericin B lipid complex are published, definitive conclusions regarding their relative efficacies for mucormycosis cannot be made. For now, the concordance of pharmacokinetic data, animal model data, and retrospective clinical data all support the first line use of high-dose liposomal amphotericin B for mucormycosis, particularly for cases of central nervous system disease, with amphotericin B lipid complex serving as a reasonable alternative agent. Therefore, a rational approach to the treatment of life-threatening mucormycosis infections is emergent surgical consultation followed by immediate initiation of liposomal amphotericin B at 5–10 mg/kg/d for non-CNS disease, or possibly higher (e.g., 10–15 mg/kg/d) for CNS disease (Table 5).

Voriconazole is not active against the Mucorales *in vitro* [294]. Conversely, the recently FDA-approved drug, posaconazole, and the investigational drug, ravuconazole, have promising *in vitro* activity against agents of mucormycosis [294,295]. Van Burik *et al*. reported a 60% 'response rate' to salvage posaconazole in polyene-experienced patients with mucormycosis [296]. However, it is important to emphasize that these patients had all been treated with polyene therapy, and in many cases with lipid polyenes which have very long tissue half lives. Therefore, this salvage therapy was more representative of combination therapy. Furthermore, the complete response rate was only 15% [296]. In light of the fact that murine models of disseminated mucormycosis found posaconazole to be significantly less efficacious than amphotericin B deoxycholate [297,298], and to result in few long term surviving animals, the precise utilization of posaconazole for this disease remains unclear and warrants study in prospective trials.

Echinocandins have minimal activity against the agents of mucormycosis when tested *in vitro* by standard techniques [299,300]. However, it is now known that *R. oryzae* expresses the target enzyme for caspofungin [301], and in a murine model of disseminated mucormycosis, caspofungin did have limited activity against *R. oryzae* [301]. Furthermore, it has recently been reported that in diabetic ketoacidotic mice with disseminated *R. oryzae* infection, combination of caspofungin (1 mg/kg/d) plus amphotericin B lipid complex (5 mg/kg/d) was synergistic [290]. While either therapy alone mediated no survival benefit, the combination significantly improved survival (50% survival for the combination vs 0% for placebo, caspofungin alone, or amphotericin B lipid complex alone). These data suggest that echinocandins may have a role as a second agent, especially in combination with a polyene, in serious cases of mucormycosis. More study of the utility of echinocandins is needed in this setting.

Iron chelation therapy

It has been known for two decades that patients in renal failure treated with the iron chelator, deferoxamine, have a markedly increased incidence of invasive mucormycosis [302]. However, it is now clear that iron chelation is not the mechanism by which deferoxamine enables mucormycosis infections. To the contrary, while deferoxamine is an iron chelator from the perspective of the human host, *Rhizopus* actually utilizes deferoxamine as a siderophore to supply previously unavailable iron to the fungus [303,304].

The central role of iron metabolism in the pathogenesis of mucormycosis suggests the possibility of utilizing effective iron chelators as adjunctive antifungal therapy. In fact, two experimental iron chelators have been studied *in vitro* against *R. oryzae* [302]. In contrast to deferoxamine, these other iron chelators did not allow the organism to take up iron, and did not support its growth *in vitro* in the presence of iron. Furthermore, while deferoxamine significantly worsened disseminated *R. oryzae* infection in guinea pigs,

one of the other chelators had no impact on the *in vivo* infection and the other chelator, deferiprone, more than doubled the mean survival time [302]. In more recent experiments with a diabetic ketoacidotic murine model, treatment with deferiprone markedly improved survival from disseminated mucormycosis, although the drug had a very narrow therapeutic window [305]. This survival benefit was reversed with administration of free iron, confirming that iron chelation was the mechanism of protection. Deferiprone is approved for the treatment of iron-overload in India and Europe, and is available on a compassionate use basis in the US and Canada.

Deferasirox (Exjade, Novartis) is a new orally available iron chelator that was recently approved by the US Food and Drug Administration (FDA) for the treatment of iron overload in transfusion-dependent anemias [306]. Recently we utilized deferasirox as a salvage agent in a patient with advanced rhinocerebral mucormycosis who had radiographic evidence of progressive brainstem disease despite extensive surgical debridement and 7 months of maximal tolerated doses of liposomal amphotericin B [307]. After only 7 days of treatment with desferasirox, a virtually complete reversal of the disease progression was noted. Several weeks later all antifungal therapy was stopped, and the patient has remained asymptomatic and disease-free for more than a year. The promising results of these experiments and case report advocate for further study of iron chelation as an adjunctive therapy for mucormycosis.

The role of surgery

Mucormycosis is frequently rapidly progressive and antifungal therapy alone is often inadequate to control the infection. Furthermore, the hallmark angioinvasion, thrombosis, and tissue necrosis of this disease results in poor penetration of anti-infective agents to the site of infection. Therefore, even if the causative organism is susceptible to the treating antifungal agent *in vitro*, the antifungal may be ineffective *in vivo*. Surgical debridement of infected and necrotic tissue should be performed on an urgent basis.

Published case series continue to support the need for surgical debridement to optimize outcomes. For example, in a case series totaling 49 patients with rhinocerebral mucormycosis, the mortality was 70% in cases treated with antifungal agents alone, versus 14% in cases treated with antifungal agents plus surgery [308,309]. Similarly, in a combined series of rhinocerebral, cutaneous, and pulmonary mucormycosis, 11/17 (65%) of patients treated with surgery plus antifungal agents survived the infection, compared to 0/7 (0%) of patients treated with antifungal agents alone [264]. A variety of other series of pulmonary and non-pulmonary mucormycosis cases have concurred with this finding [249,261,273,310–312]. Clearly there is the potential for selection bias in these case series, as patients who do not undergo surgery may have fundamental differences in severity of illness or co-morbidities. Nevertheless, the observational clinical data supports the concept that surgical debridement is necessary to optimize cure rates.

Fusarium

Epidemiology

Fusarium infections are less common than *Aspergillus* infections, even in the transplant setting. For example, in one study, *Fusarium* infections were ~9-fold less common than *Aspergillus* infections in patients status post HSCT [313]. In a separate multi-center study of HSCT recipients, the incidence of fusariosis varied from 1.4–2 or 5–20 cases per 1,000 autologous or allogenic transplants, respectively, depending on the degree of human leukocyte antigen mis-match [314].

The most common species causing fusariosis infections are *F. moniliforme*, *F. solani*, and *F. oxysporum*. Increases in transplantation and use of immune suppressing agents have led to a dramatic rise in incidence of nosocomial *Fusarium* infections [314]. For example, the number of probable or proven fusariosis in HSCT recipients at one center exceeded the number of mucormycosis infections over the same time period [314]. As with invasive aspergillosis, the rise in fusariosis incidence may be partially attributable to the routine use of fluconazole prophylaxis post-transplant [9]. Colonized water systems in the hospital environment have been identified as reservoirs of *Fusarium*, as aerosolization and patient-to-patient spread subsequently may lead to infections [315].

Risk factors

Although rare, fusariosis in immunocompetent hosts is well described and generally manifests as soft tissue or mucosal infection after direct inoculation of the mold into the skin or eye (i.e., keratitis due to contact lenses) by trauma, foreign body, or burns [316], or as onychomycosis [316]. In contrast, invasive fusariosis is essentially a nosocomial disease of the immune-compromised. The severity and duration of immune suppression appears to be the most important factors in creating risk for fusariosis, and HSCT patients are at highest risk. A retrospective review of fusariosis in

HSCT patients identified a trimodal distribution, with peaks prior to engraftment, within 100 days post engraftment, and after 1 year post engraftment [314]. The highest incidence occurred within 100 days [314]. This finding confirmed prior observations in which approximately 30% of fusariosis was diagnosed within 40 days of transplant, and nearly 80% within 180 days [9]. As with other invasive molds, neutropenia is the major risk factor for development of early disease (within 30 days of transplant), while GVHD predicts a later presentation (>30 days) [314]. By contrast, fusariosis is rare among solid-organ transplant patients, and has a much lower mortality rate in this population [317].

Diagnosis

Invasive *Fusarium* infections often present with skin manifestations, most commonly with purpuric nodules with central necrosis [318]. In one series, 75% of fusariosis cases presented with dissemination and skin lesions [314]. Histopathologic evidence of vascular invasion is typical in this setting [319]. On histopathological examination, the organism appears as hyaline, acute-branching, septate hyphae that may be indistinguishable from *Aspergillus* species [316]. Unlike other invasive molds, *Fusarium* grows from blood cultures in >40–75% of cases [318,320]. Besides the skin, common sites of involvement include the lung and sinuses [314].

Treatment and prognosis

Fusarium tends to be much less susceptible to polyenes *in vitro* than other molds such as *Aspergillus*, and breakthrough infections have occurred on polyene therapy [321]. In addition, fluconazole, itraconazole, and flucytosine are not active against *Fusarium* species. Successful outcomes have occurred with voriconazole, and voriconazole is increasingly recognized as the drug of choice for the treatment of these infections, either with or without the addition of a polyene. As well, a recent retrospective review of posaconazole as salvage therapy for fusariosis suggested that it may be useful for refractory disease [322].

Reversal of the underlying immune suppression is crucial in the therapeutic approach of fusariosis. Specifically, a reduction of duration of neutropenia by administration of colony stimulating factors, or, possibly by white cell transfusions, and a reduction or elimination of corticosteroids, should be attempted if feasible [295,314,320,323]. Not surprisingly given the relative dearth of active antifungals against the organism, outcomes in fusariosis have been dismal. In a report of 259 patients, most with underlying malignancies, mortality was 66% overall but was 100% in the persistently neutropenic group [319]. In another series, the mortality at 90 days after diagnosis was 79%, and was 100% in patients with both neutropenia and corticosteroid therapy [150]. In select cases, surgical management and/or topical antifungal therapy may help reduce morbidity and mortality.

Other invasive molds

Among other invasive fungi, *Scedosporium apiospermum* (teleomorph *Pseudallescheria boydii*) and *S. prolificans* may be seen in immunocompromised hosts. While *S. apiospermum* is implicated in a fraction of subcutaneous mycetoma cases in immunocompetent persons, these infections are typically invasive into deeper tissues in transplanted hosts, with clinical presentations, treatment, and prognosis similar to that of *Fusarium* species [9]. Disseminated presentations are most common but CNS involvement is also seen. These infections are more common in recipients of HSCT than solid-organ transplants [324].

S. apiospermum is susceptible to the triazoles voriconazole, posaconazole, and ravuconazole *in vitro*, while showing higher MIC's to amphotericin B, itraconazole, and nystatin. Unfortunately, *S. prolificans* tends to be resistant to virtually every antifungal currently available, and represents a particularly difficult therapeutic challenge [325]. The outcomes of infections caused by *S. prolificans* are particularly poor. Of note, several recent case reports have described successful outcomes following prolonged combination therapy with voriconazole and terbinafine [326–328]. Given the abysmal outcomes with prior antifungal strategies, combination voriconazole plus terbinafine therapy may be a reasonable strategy for the treatment of *S. prolificans* infections.

Other molds implicated in nosocomial infections include *Acremonium, Paecilomyces, Cladophialophora, Cladosporium*, and many other hyalohyphomyces and dematiaceous fungi. These infections occur in similar patient populations and can be diagnosed and treated in a similar fashion to *Scedosporium* spp.

Conclusions

Invasive fungal infections are increasingly frequent nosocomial problems. They tend to affect patients with compromised host defense mechanisms, resulting in high morbidity and mortality despite antifungal therapy. Given that the underlying risk factors for invasive fungal infections are increasingly common in

the US and globally in countries with advanced medical technologies, it is anticipated that the incidence of these infections will continue to increase in the coming decades.

Early initiation of therapy is critical to mitigating the risk of mortality, requiring maintenance of a high index of clinical suspicion. Combination therapy should be considered for severe disease in compromised hosts. Further research is desperately needed to improve early diagnostic modalities, and to develop new therapeutic strategies. Perhaps most important would be the development of new strategies to prevent these deadly infections from occurring in the first place.

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