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Review of epidemiology, diagnosis, and treatment of invasive mould infections in allogeneic hematopoietic stem cell transplant recipients

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Abstract

Invasive mould infections are a major cause of morbidity and mortality in hematopoietic stem cell transplant recipients (HSCT). Allogeneic HSCT recipients are at substantially higher risk than autologous HSCT recipients. Although neutropenia following the conditioning regimen remains an important risk factor for opportunistic fungal infections, most cases of invasive mould infection in allogeneic HSCT recipients occur after neutrophil recovery in the setting of potent immunosuppressive therapy for graft-versus-host disease. Invasive aspergillosis is the most common mould infection. However, there has been an increased incidence of less common non-*Aspergillus* moulds that include zygomycetes, *Fusarium* sp., and *Scedosporium* sp. Reflecting a key need, important advances have been made in the antifungal armamentarium. Voriconazole has become a new standard of care as primary therapy for invasive aspergillosis based on superiority over amphotericin B. There is significant interest in combination therapy for invasive aspergillosis pairing voriconazole or an amphotericin B formulation with an echinocandin. There have also been advances in novel diagnostic methods that facilitate early detection of invasive fungal infections that include galactomannan and beta-glucan antigen detection and PCR using fungal specific primers. We review the epidemiology, diagnosis, and management of invasive mould infection in HSCT, with a focus on allogeneic recipients. We also discuss options for prevention and early treatment of invasive mould infections.

Key words: *Aspergillus*, *Fusarium*, graft-versus-host disease, mould, stem cell transplant, zygomycete

Introduction

Invasive mould infections are a principal cause of morbidity and mortality in hematopoietic stem cell transplant (HSCT) recipients. Allogeneic HSCT recipients have a greater risk of invasive mould infections compared to autologous HSCT recipients. The risk for invasive mould infections in allogeneic HSCT recipients follows a time-line. Neutropenia resulting from the conditioning regimen is the principal risk factor for fungal infections during the early transplant period (first 30 days). Immunosuppressive therapy for graft-versus-host disease (GVHD) is the major risk factor in the later transplant period.

Invasive aspergillosis is the most common opportunist mould infection. However, the emergence of less common but medically important fungal pathogens has contributed to the rate of morbidity and mortality in allogeneic HSCT recipients. These pathogens include septate filamentous fungi (e.g., *Fusarium* sp., *Acremonium* sp., *Scedosporium* sp., dark-walled moulds) and aseptate or hyposeptate zygomycetes [1, 2]. Similar to

infection by *Aspergillus* sp., risk factors for these less common mould infections are principally related to the duration of neutropenia and to the intensity of immunosuppressive therapy for GVHD. Marr et al. [3] noted an increased incidence of infections by non-fumigatus *Aspergillus* species, zygomycetes, and *Fusarium* and *Scedosporium* species in allogeneic HSCT recipients, particularly in patients with multiple stem cell transplants. Such patients would be among the most highly immunocompromised due to relapsed malignancy, multiple prior cycles of cytotoxic regimens, and immunosuppressive therapy for GVHD.

We will review the epidemiology, diagnosis, and management of invasive mould infection in HSCT, with a focus on allogeneic recipients. Advances in the antifungal armamentarium and novel diagnostic methods for early detection of invasive fungal infections will be reviewed. We will also discuss options for prevention and early treatment of invasive mould infections. The subject of immune augmentation for fungal infections has been comprehensively reviewed in several recent publications [4–8] and will therefore not be addressed in the current paper.

Invasive aspergillosis after hematopoietic stem cell transplantation

Epidemiology of invasive aspergillosis

The spectrum of pathogens to which HSCT recipients are most susceptible follows a time-line corresponding to the predominant immune defects observed at different periods. In the first month after HSCT, neutropenia and mucosal toxicity from the conditioning regimens are the principal host defense defects. These patients are at risk for the same spectrum of fungal pathogens (principally *Candida* sp. and filamentous fungi) that afflict non-transplant patients with hematologic malignancies treated with potent cytotoxic therapy.

Defects in cell-mediated immunity persist for several months even in uncomplicated allogeneic transplant recipients, thus predisposing these patients to a variety of opportunistic fungal infections. In addition to quantitative T-cell deficiencies, loss of T-cell receptor diversity is observed [9]. In the absence of GVHD, T-cell subsets and responses normalize by 1 year after HSCT.

In a recent prospective, multicenter surveillance study, the cumulative incidence of invasive aspergillosis at 12 months was 0.5% after autologous HSCT, 2.3% after allogeneic HSCT from an human leukocyte antigen (HLA)-matched related donor, 3.2% after transplantation from an HLA-mismatched related donor, and 3.9% after transplantation from an unrelated donor [10]. HLA-antigen disparity increases the frequency and severity of GVHD; immunosuppressive therapy to control GVHD disables immune reconstitution and predisposes to infectious complications. Several studies have reported the predominance of aspergillosis cases occurring in the post-engraftment rather than in the neutropenic period in allogeneic HSCT recipients [11–18], with immunosuppressive therapy for GVHD being a principal risk factor.

Marr et al. [17] retrospectively evaluated risk factors for invasive aspergillosis at different time points after allogeneic HSCT. Factors that were associated with an increased risk of invasive aspergillosis after engraftment included receipt of T cell-depleted or CD34-selected stem cell products, treatment with corticosteroids, neutropenia, lymphopenia, GVHD, cytomegalovirus (CMV) disease, and respiratory viral infections. Very late invasive aspergillosis (more than 6 months after transplantation) was associated with chronic GVHD and CMV disease. Late onset invasive aspergillosis is also a major cause of mortality in non-myeloablative allogeneic HSCT recipients, with acute and chronic GVHD, recurrent neutropenia, relapse of malignancy, and cytomegalovirus disease being the principal risk factors [19–21].

There are three likely reasons for the increased proportion of invasive aspergillosis in the post-engraftment period: (1) shortening of the duration of neutropenia period as a result of infusion of larger numbers of myeloid progenitors and treatment with hematopoietic growth factors; (2) increased proportion of unrelated donors and HLA-mismatched transplants which predispose to GVHD and increased requirement for immunosuppression; and (3) increased proportion of patients surviving beyond the early transplant period.

Cord blood transplant (CBT) recipients have a higher risk of infections than other allograft

recipients during the early transplant period because of slower myeloid engraftment [17]. We have also noted a significant frequency of opportunistic infections after day 100 in CBT recipients, including invasive aspergillosis [22]. Invasive aspergillosis occurred more than 3 years after CBT in 2 of the 15 long-term survivors, and was associated with corticosteroid therapy for GVHD.

Among patients with invasive aspergillosis, allogeneic HSCT recipients have the poorest response to antifungal therapy [23–25]. The likelihood of mortality is in part related to the intensity of immunosuppressive therapy for GVHD [18, 26]. There has been a significant expansion in immunosuppressive agents to treat GVHD, which include antibodies targeted toward specific lymphocyte subsets, inhibit T-cell co-stimulatory pathways, and anti-cytokine antibodies. TNF-α is a principal mediator of acute inflammation to pathogens, and stimulates the activation and recruitment of neutrophils and monocytes. Infliximab used in HSCT recipients with severe GVHD is associated with an increased risk of invasive filamentous fungal infections [27, 28].

Autologous transplants are associated with less infectious complications than allogeneic transplants. However, CD34 enrichment of autografts leads to a significant reduction in T-cells, natural killer cells, and monocytes, compared with unmanipulated autografts – which adversely affect immune reconstitution and thus increase the risk for infections. Recipients of CD34-enriched autografts appear to have a risk of opportunistic infections comparable to allogeneic HSCT recipients [29].

Pathology of invasive aspergillosis

Animal models of invasive aspergillosis and experience in patients suggest that *Aspergillus* infection during neutropenia is pathologically and immunologically distinct from infection in the absence of neutropenia in the setting of potent immunosuppressive regimens used to treat GVHD. Berenguer et al. [30] reported that in profoundly neutropenic rabbits challenged with *Aspergillus fumigatus*, pulmonary lesions consisted predominantly of coagulative necrosis, intraalveolar hemorrhage, and scant mononuclear inflammatory infiltrate. In contrast, pulmonary foci in rabbits treated with cyclosporine plus corticosteroids (modeling the immunosuppression in patients undergoing hematopoietic and solid organ transplantation) consisted mainly of neutrophilic and monocytic infiltrates, inflammatory necrosis, and scant intraalveolar hemorrhage. This difference has in general also been observed in patients with invasive aspergillosis, reflecting the fact that patients with chemotherapy-induced neutropenia and transplant recipients treated with cyclosporine and corticosteroids are two immunologically distinct populations. However, there are sparse data about the histopathology of invasive aspergillosis (and other mould infections) in HSCT recipients after neutrophil recovery.

In our single center review of invasive mould infections in allogeneic HSCT recipients, 21 of 22 cases (95%) were diagnosed after neutrophil recovery [18]. All had received systemic corticosteroids within one month prior to diagnosis of mould infection. *Aspergillus* species were isolated in 18 (82%) cases. In contrast to animal studies, the predominant lung histopathology was coagulative necrosis with sparse inflammation similar to the histology associated with neutropenic hosts. Hyphal angioinvasion was observed in some of these cases (Figure 1). In contrast to our results, Chamilos et al. [31] reported in a large autopsy series that, a low fungal burden and high inflammatory pattern characterized invasive pulmonary aspergillosis associated with GVHD whereas a high fungal burden and coagulative necrosis predominated during neutropenia. We speculate that the predominance of coagulative necrosis observed in non-neutropenic allogeneic HSCT recipients in our series may reflect the high doses of corticosteroids used to treat GVHD, which may have disabled leukocyte trafficking and hyphal killing. Understanding the pathophysiology of invasive mould infections in relation to host factors will be important in designing novel therapeutic strategies, such as immune augmentation.

Treatment of invasive aspergillosis

Voriconazole has become a new standard of care for invasive aspergillosis (Table 1). In an open-label, multicenter randomized trial of primary therapy for invasive aspergillosis, voriconazole was more effective than amphotericin B (53% vs. 32% of subjects had a complete or partial response) and was associated with improved

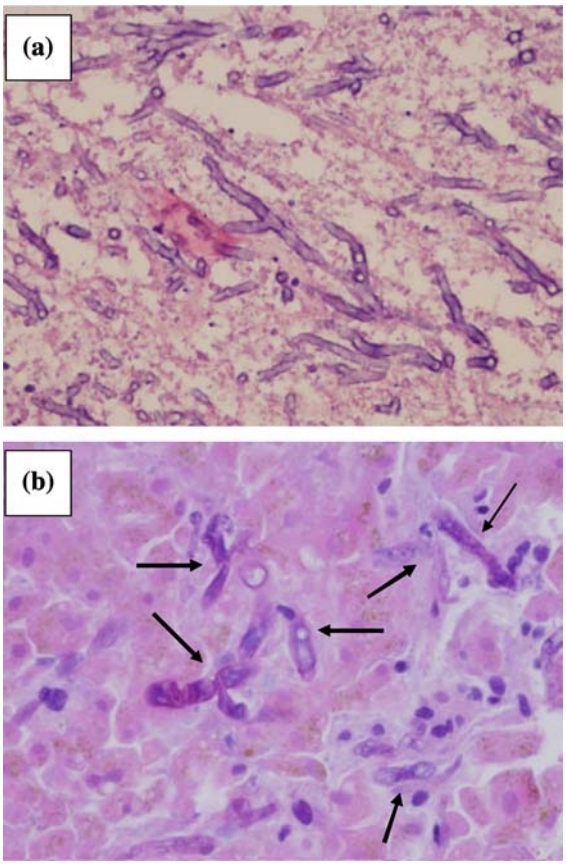

Figure 1. (a) Invasive aspergillosis of the heart causing extensive myocardial necrosis in an allogeneic HSCT recipient. Note the characteristic septated acute-angle branching hyphae (H&E, 400×). (b) Hepatic mucormycosis in an allogeneic HSCT recipient. The aseptate, wide, ribbon-like appearance and 90-degree angle branching of hyphae (arrows) are suggestive of zygomyctes (H&E, 400×). Confirmation by culture is required for definitive diagnosis.

survival at 12 weeks (71% vs. 58%, respectively) [25]. Among neutropenic patients, the success rate in the voriconazole arm was 51%, which was superior to the amphotericin B arm [25]. In a non-comparative study of 116 patients with invasive aspergillosis in which voriconazole was given either as initial (52%) or salvage (48%) therapy, a complete or partial response occurred in 48% of patients, with a more favorable prognosis in the initial therapy group [23]. In both the randomized and non-comparative studies, the poorest prognosis was observed in extrapulmonary aspergillosis and in allogeneic HSCT recipients. In a retrospective analysis of 86 patients with CNS aspergillosis treated with voriconazole either as primary or salvage therapy, 35% had a complete or partial response [32]. This success rate compares very favorably to previous series in which the frequency of successful responses to amphotericin B was almost nil [33]. Voriconazole appears to have comparable safety and efficacy in children with invasive mould infections compared to adults [34]. Based on the strength of this database, we advise voriconazole as first-line therapy for invasive aspergillosis.

Aspergillus fumigatus followed by *Aspergillus flavus* and *Aspergillus terreus* are the most common species causing invasive disease in neutropenic patients and following HSCT. *Aspergillus terreus* has been observed with increased frequency at several cancer centers, and is notable for being resistant to amphotericin B [35]. In a multivariate analysis of 83 cases of *A. terreus*, treatment of voriconazole was associated with improved survival compared with amphotericin B [35].

Echinocandins have not been evaluated as initial monotherapy for invasive aspergillosis in clinical trials. Caspofungin as salvage therapy in patients with invasive aspergillosis led to a favorable response in 37 (45%) of 83 patients [36]. There has been significant interest in combination antifungal therapy pairing an echinocandin with either an amphotericin B preparation or an azole with activity against *Aspergillus* species. The rationale is that echinocandins target a unique site (the beta-glucan constituent of the fungal cell wall) distinct from the polyenes and azoles that target the fungal cell membrane. The combination of an echinocandin with an azole or amphotericin B has shown neutral to synergistic activity in vitro. Enhanced efficacy of combination regimens pairing an echinocandin with either an azole or an amphotericin B formulation was observed in some animal models of invasive aspergillosis [37–40] but not in others [41–43].

The combination of caspofungin and liposomal amphotericin B as salvage therapy led to a favorable outcome in approximately 40–60% of patients with invasive aspergillosis, though these series included cases of 'possible' aspergillosis [44, 45]. Marr et al. [46] reported a survival advantage of voriconazole plus caspofungin compared to voriconazole alone in a retrospective analysis of salvage therapy for invasive aspergillosis. This database, though encouraging, involved small numbers of patients and the two groups of patients

Table 1. Antifungal agents used as prophylaxis and therapy in hematopoietic stem cell transplant recipients

Antifungal agent	Comments
Azoles	• Inhibit synthesis of ergosterol, a key fungal cell membrane constituent • Inhibit cytochrome P450 isoenzymes, leading to ↓ clearance of several drugs; adjustment of doses of drugs may be required (e.g., calcineurin inhibitors) and others are contraindicated
Fluconazole	• Acceptable alternative to amphotericin B for candidemia at dose of 400–800 mg/d [91] • Broad range of MICs to *C. glabrata*; *C. krusei* is resistant • Prophylaxis in high-risk patients (e.g., acute leukemia during neutropenia, hematopoietic transplantation) • Maintenance therapy for cryptococcal meningitis • Inactive against filamentous fungi
Voriconazole	• New standard of care for invasive aspergillosis based on superiority over amphotericin B desoxycholate as primary therapy for invasive aspergillosis [25] • Similar efficacy but less infusional and nephrotoxicity compared with strategy of amphotericin B followed by fluconazole in randomized study of primary therapy for candidemia and invasive candidiasis [92] • Intravenous formulation should be used with caution in patients with pre-existing significant renal impairment because of potential for cyclodextrin vehicle to accumulate in serum and worsen renal function • Visual side effects are common, but are usually transient and rarely require discontinuing therapy
Itraconazole	• 63% success rate in compassionate use trial for invasive aspergillosis [93] • Used in some centers as prophylaxis in patients with leukemia receiving chemotherapy and in allogeneic HSCT recipients • FDA-approved as empirical antifungal therapy for neutropenic fever • Negative cardiac inotropic effects; should not be used in patients with significant cardiac systolic dysfunction • Intravenous formulation should be used with caution in patients with pre-existing significant renal impairment because of potential for cyclodextrin vehicle to accumulate in serum and worsen renal function
Posaconazole	• Broad spectrum oral antifungal azole available in U.S. through limited access protocol (Schering-Plough); approved in Europe for refractory invasive aspergillosis, fusariosis, and other fungal infections • Large database as salvage therapy in several opportunistic fungal infection; 42% success rate in patients with invasive aspergillosis refractory or intolerant to standard therapy [48] • Superior efficacy over comparator regimens as prophylaxis in a randomized study of allogeneic HSCT recipients with significant GVHD [88] and in a randomized study of patients receiving cyotoxic chemotherapy for acute leukemia [94]
Ravuconazole	• Phase I/II study completed in HSCT recipients
Amphotericin B formulations	• Broad spectrum of antifungal activity • Significant infusional toxicity, nephrotoxicity, and electrolyte wasting • Lipid formulations have at least equal efficacy and less toxicity compared to amphotericin B desoxycholate
Liposomal amphotericin B	• ↓ proven breakthrough fungal infections and ↓ infusion- and nephrotoxicity vs. Amb-D as empirical therapy for persistent neutropenic fever [95]; ↓ infusion-nephrotoxicity vs. amphotericin B lipid complex as empirical therapy [96] • No randomized studies as therapy for invasive aspergillosis
Amphotericin B lipid complex	• Large patient registry on therapy for invasive candidiasis, aspergillosis and other opportunistic fungi [64, 97] • No randomized studies published in peer-reviewed journals
Amphotericin B colloidal dispersion	• Similar efficacy, ↓ nephrotoxicity, but ↑ infusion toxicity vs. Amb-D in a randomized study of primary therapy for invasive aspergillosis [98]
Echinocandins	• Class of antifungal peptides that inhibit synthesis of Beta-glucan, a fungal cell wall constituent • Active against *Candida* and *Aspergillus* species • Infrequent infusion-related events and not nephrotoxic • Data supporting pairing echinocandin with an azole or amphotericin B formulations for invasive aspergillosis are derived from in vitro and some (but not all) animal studies, and a limited patient database involving non-randomized studies
Caspofungin	• FDA-approved as salvage therapy for invasive aspergillosis • Retrospective analysis showed increased survival of voriconazole + caspofungin vs. voriconazole alone as salvage therapy for invasive aspergillosis [46] • Similar efficacy but less infusional and nephrotoxicity compared with amphotericin B in randomized study of primary therapy for candidemia and invasive candidiasis [99] • Comparable efficacy and ↓ toxicity vs. liposomal amphotericin B as empirical therapy for neutropenic fever [100]

Table 1. Continued

Antifungal agent	Comments
Micafungin	• Superior efficacy compared with fluconazole as prophylaxis in HSCT recipients [85] • Limited database on therapy for invasive aspergillosis • Similar efficacy compared with liposomal amphotericin B in randomized study of primary therapy for candidemia and invasive candidiasis [101]
Anidulafungin	• Superior efficacy compared with fluconazole in randomized study of primary therapy for candidemia and invasive candidiasis [102] • No published database on invasive aspergillosis
Flucytosine	• Pyrimidine analogue with dose- and duration-dependent marrow and gastrointestinal toxicity • Monitoring of serum levels and adjustment of dosing for azotemia required • Limited data on pairing 5-flucytosine with an azole or amphotericin B formulation as therapy for invasive aspergillosis

evaluated were non-contemporaneous; therefore, other host and infection-related factors may have influenced the outcome. A recent non-comparative study of caspofungin combined with other mould-active drugs as salvage therapy for invasive aspergillosis resulted in a success rate of 55% [47], which compared favorably with a prior study of salvage therapy with caspofungin alone (45% success) [36]. A randomized, prospective study is required to definitively assess the benefit of combination antifungal therapy in invasive aspergillosis.

Posaconazole has been effective as salvage therapy against a broad spectrum of invasive fungal infections [48]. Forty-two percent of patients with invasive aspergillosis that was refractory or who had intolerance to standard antifungal therapy had a complete or partial response to posaconazole [48]. Posaconazole has been approved in the European Union for treatment of invasive aspergillosis and certain other invasive fungal infections refractory to standard antifungal agents. Posaconazole has not yet been approved by the U.S. Food and Drug Administration, but is available through a compassionate use protocol from Schering-Plough.

Patients who recover from an episode of invasive aspergillosis are at risk for relapse of infection during subsequent immunosuppression [49, 50]. In patients with invasive aspergillosis prior to HSCT, antifungal therapy for more than a month and resolution of radiologic abnormalities correlate with a lower likelihood of post-transplant recurrence of infection [50]. Secondary prophylaxis with a mould active agent is advised for the entire period of immunosuppression.

Non-aspergillus mould infections

Zygomycetes

Risk factors for zygomycosis (also referred to as ''mucormycosis'') include prolonged neutropenia, corticosteroid therapy, diabetic ketoacidosis, and iron overload. Among allogeneic HSCT recipients, zygomycosis may occur early after transplant during neutropenia following conditioning, or later as a complication of GVHD. The frequency of invasive zygomycosis has increased at some centers in the setting of more frequent voriconazole usage [51–53]. Kontoyiannis et al. [53] reported that breakthrough infection while receiving voriconazole and sinusitis were both more common in cases of zygomycosis compared to aspergillosis among patients with acute leukemia and allogeneic HSCT recipients. However, some transplant centers reported an increased frequency of zygomycosis that pre-dated the availability of voriconazole [3, 54], a finding that likely reflects a greater proportion of patients with severe host defense impairment.

Zygomycosis typically manifests as rhinocerebral or pulmonary disease following inhalation of spores. In a comprehensive review of zygomycosis, Roden et al. [55] noted that 92 of 154 (60%) of patients with malignancy had pulmonary disease, whereas 222 of 337 (66%) of patients with diabetes had sinus disease. Infection due to *Cunninghamella* species and disseminated disease were independently associated with increased mortality. In rhinocerebral disease, fever, facial pain and headache are common findings. Contiguous extension may lead to orbital involvement with proptosis and

extraocular muscle paresis, involvement of hard palate, and spread to the brain. An eschar over the palate or nasal turbinates is suggestive of zygomycosis, but other filamentous fungi can produce similar findings. Occasionally, isolated primary cutaneous disease may occur, but is often a harbinger for deep soft tissue or disseminated infection in the highly immunocompromised. Histopathology showing broad aseptate or hyposeptate hyphae with 90-degree branching is suggestive of zygomycosis, though culture is required for confirmation (Figure 1).

Treatment of zygomycosis involves high dose amphotericin B (conventional or lipid formulations) plus early and aggressive surgical debridement. Posaconazole, a second generation antifungal azole, is promising as salvage therapy in zygomycosis refractory to amphotericin B formulations [56, 57].

Fusarium species

Fusarium species are soil saprophytes that have been associated with soft tissue infection, onychomycosis, and keratitis in immunocompetent hosts. With the increased number of persons treated with intensive cytotoxic therapy for acute leukemia and allogeneic HSCT, invasive and disseminated fusariosis has become more common over the past 20 years [58]. The likelihood of infection by a *Fusarium* species is substantially increased by the presence of disseminated cutaneous lesions and isolation of a mould from blood culture. Skin is as an important portal of entry for fusariosis [58]. Initial localized manifestations included onychomycosis, paronychia, and cellulitis. Early identification of localized skin disease and debridement may be life-saving. Inhalation of spores is another major portal of entry, leading to fungal sinusitis and pneumonia. *Fusarium* infection may be associated with colonization of hospital water systems [59], though the outside environment is likely to be the principal reservoir [60].

In a multicenter retrospective analysis of fusariosis, Nucci et al. [61] noted a trimodal distribution in allogeneic HSCT recipients: a first peak before neutrophil recovery, a second peak at a median of 62 days after transplantation, and a third peak more than 1 year after transplantation. The incidence of fusariosis among allogeneic HSCT recipients varied between approximately 0.5% in HLA-matched related transplant recipients to ~2% in HLA-mismatched transplant recipients (the latter are at higher risk for GVHD). Overall survival was 13%, and persistent neutropenia was the single prognostic factor for death identified by multivariate analysis.

Therapy for invasive fusariosis involves a 2nd generation antifungal azole (voriconazole or posaconazole) or a lipid formulation of amphotericin B. The database is largely derived from a small number of patients enrolled in salvage therapy protocols or retrospective analysis. Voriconazole led to a 46% success rate in invasive fusariosis [62]. Posaconazole led to a 50% positive response rate as primary and salvage therapy for invasive fusariosis [63], and has been approved in the European Union for refractory fusariosis. Amphotericin B lipid complex led to a positive response in 46% of patients with fusariosis [64]. Reversing the underlying immunodeficiency (e.g., recovery from neutropenia) is likely to be the key variable predictive of a positive outcome [61, 65].

Scedosporium species

Scedosporium apiospermum (*Pseudallescheria boydii*) and *Scedosporium prolificans* are the principal pathogenic species. In neutropenic patients, *S. apiospermum* is a virulent pathogen, which clinically and histologically resembles aspergillosis. Invasion of blood vessels leading to infarction is common. *S. apiospermum* causes sinopulmonary disease, endophthalmitis, and dissemination to the central nervous system infection. The infection can also spread directly from the skin to bone and joint. Establishing a culture diagnosis of *S. apiospermum* is important because of its frequent resistance to amphotericin B. *S. prolificans* causes a similar spectrum of disease as *S. apiospermum*, but is generally resistant to all antifungal agents. Therapy for scedosporiosis generally involves itraconazole, voriconazole, or posaconazole. Perfect et al. [62] reported a success rate of voriconazole of 30% in patients with scedosporiosis. Surgical resection of localized lesions is advised when feasible.

Dematiaceous (dark-walled) moulds

Dark-walled moulds (phaeohyphomycetes) contain melanin in their cell walls that imparts a

brown or olive-green pigment in culture. In immunocompromised patients, soft tissue infection, sinusitis, central nervous system infection, pneumonia, fungemia, and disseminated disease are observed. Subcutaneous infection is most frequently caused by *Alternaria* species. *Bipolaris*, *Cladophialophora* (*Xylohypha* or *Cladosporium*) *bantiana*, *Wangiella*, and *Dactylaria* species have a strong predisposition to cause central nervous system disease.

Therapy in immunocompromised patients involves surgical excision of localized disease when feasible, and systemic antifungal therapy. Sensitivity to amphotericin B is variable and clinical failures have been reported. Itraconazole has been the preferred agent for phaeohyphomycosis [66]. Voriconazole and posaconazole have in vitro activity against dark-walled moulds, and are also viable options.

Diagnosis of invasive mould infections

Cultures

Diagnosis of invasive mould infections remains difficult. Bronchoalveolar cultures have approximately 50% sensitivity, and definitive diagnosis often requires a thoracoscopic or open lung biopsy [67]. Blood cultures are almost never positive except for *Fusarium* sp. Hence, there is a need for additional modalities to facilitate early diagnosis.

Chest CT scans

CT scanning of the chest may facilitate early detection of aspergillosis. A CT scan may show peripheral or subpleural nodules inapparent on plain chest radiographs. One or more nodules are the most common finding on chest CT in invasive pulmonary aspergillosis in patients with neutropenia and HSCT recipients [68]. The ''halo sign'' is a characteristic chest CT feature of angioinvasive organisms [69]. The hazy alveolar infiltrates appear to correspond to regions of ischemia, and are highly suggestive of invasive aspergillosis [69]. In one study, chest CT scans performed in patients with persistent neutropenic fever facilitated detection of pulmonary aspergillosis, leading to early initiation of therapy and improved outcomes [70].

Caillot et al. [71] performed sequential CT scans on patients with neutropenia and invasive aspergillosis. Despite effective antifungal treatment leading to a positive clinical response in most patients, the median volume of lesions increased four-fold during the first week of therapy and remained stable during the second week. An increase in size of pulmonary lesions within the first week did not predict a negative response to therapy. Halo signs were very common at diagnosis but decreased during the first week of infection as the frequency of the air crescent sign increased. This study has implications in interpreting the results of salvage therapy or compassionate use studies in which patients with invasive aspergillosis could be enrolled after only 7 days of standard antifungal therapy if the size of pulmonary lesions were unchanged or increased.

Laboratory markers

The following laboratory markers are the principal diagnostic adjuncts of invasive mould infections: (1) galactomannan; (2) beta-glucan, and (3) PCR. Galactomannan and beta-glucan are fungal cell wall constituents that can be detected in serum during systemic infection. Galactomannan may be detected in bronchoalveolar lavage and cerebrospinal fluid in cases of pulmonary and central nervous system aspergillosis, respectively. Galactomannan is specific for invasive aspergillosis whereas beta-glucan and PCR (depending on the selection of primers) can be used to detect other fungal infections.

Each of the markers has advantages and potential pitfalls. The galactomannan test, which relies on a sensitive double sandwich ELISA, has produced variable results in terms of sensitivity and specificity. In the best scenarios, Maertens et al. [72] obtained serial serum galactomannan levels from neutropenic and HSCT patients at high risk for aspergillosis. The positive and negative predictive values were 87.5% and 98.4%, respectively. All proven cases of invasive aspergillosis, including 23 cases confirmed after autopsy only, had been detected before death, although serial sampling was necessary to maximize detection. Prospective serial monitoring of galactomannan antigenemia in allogeneic HSCT recipients yielded positive and negative predictive values of 94.4% and 98.8%, respectively, and antigenemia preceded

radiographic findings by more than a week in 80% of cases of invasive aspergillosis [73].

Herbrecht et al. [74] evaluated the galactomannan antigenemia assay in patients at risk for invasive aspergillosis. The sensitivity was 64.5% in cases of definite invasive aspergillosis. The positive predictive value (PPV) varied among different patient groups, and had the lowest values when used as a surveillance tool in patients with persistent neutropenic fever (PPV = 7.1%) and in HSCT recipients (PPV = 10%); the negative predictive value was 100% in both groups.

The galactomannan assay was evaluated using 1890 blood samples from 170 patients at high risk for invasive mould infection from three major cancer centers in North America. Using a lower cut-off (0.5 units) than the European studies, the galactomannan assay identified 25 of 31 patients with invasive aspergillosis (81% sensitivity), and had a specificity of 89% (http://www.fda.gov/bbs/topics/NEWS/2003/NEW00907.html). The FDA recently approved the Platelia *Aspergillus* enzyme immunoassay (Bio-Rad Laboratories, Redmond, WA).

Several variables can affect the performance of the galactomannan assay [75, 76] that likely account for the differences in results in these and other prospective studies. The sensitivity of the assay is reduced by concomitant use of mould-active antifungal agents [77, 78]. False positive results may be more common in children and allogeneic HSCT recipients [74]. Concomitant piperacillin/tazobactam is known to cause false positive galactomannan results [79, 80].

The beta-glucan assay has recently received FDA approval for presumptive diagnosis of invasive fungal infections. Among patients with acute myeloid leukemia and myelodysplastic syndrome, the assay was highly sensitive and specific in detecting early invasive fungal infections, including candidiasis, fusariosis, trichosporonosis, and aspergillosis [81]. The database on beta-glucan surveillance testing in HSCT recipients is preliminary and merits further study [82]. In one study, prospective monitoring of patients with cancer using the serum beta-glucan assay had increased sensitivity compared to serum galactomannan, but the galactomannan test may be more specific [83]. Both the serum galactomannan and beta-glucan assays have been accepted as diagnostic adjuncts of invasive fungal infections in the revised European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) consensus criteria (De Pauw B et al. Oral presentation, ICAAC, Dec., 2005, Washington, DC).

PCR-based detection of invasive mould infections is another promising tool for early diagnosis, though the specific primers and conditions vary in published series. Because of the lack of standardization and inter-laboratory validation of PCR methods, PCR-based detection of fungal infection is still considered an investigational tool. Additional prospective studies are required to define which diagnostic methods – or combination of methods – confer optimal sensitivity and specificity in detecting early mould infection.

Prevention and early treatment of invasive mould infections

Four general strategies of preventing and treating patients at high risk for invasive fungal infections include: (1) prophylaxis; (2) empirical antifungal therapy; (3) pre-emptive antifungal therapy and (4) treatment of established fungal infections.

In the prophylactic mode, the antifungal agent is initiated at a period of high risk of infection (e.g., at the onset of neutropenia) to prevent fungal infections. A standard definition of empirical antifungal therapy involves initiation or modification of an existing antifungal regimen on the basis of persistent neutropenic fever (generally 4–7 days) without a known source and unresponsive to appropriate antibacterial agents. The concept of empirical antifungal therapy established in the 1970s and 1980s was principally based on early treatment of occult invasive fungal infections with conventional amphotericin B. Because of its toxicity, amphotericin B was more likely to be used as empirical therapy for neutropenic fever rather than in a prophylactic mode that would entail treating a larger pool of patients at risk for fungal infection and over a longer period. With the widespread use of fluconazole in the 1990s as prophylaxis in high risk patients with acute leukemia and HSCT recipients, the incidence of invasive candidiasis decreased, but invasive mould infections became an increasing cause of mortality [84]. Empirical therapy for neutropenic fever principally involved initiation of amphotericin B to increase the spectrum of activity to include

moulds, but at the expense of greater toxicity. Indeed, only a minority of patients with persistent neutropenic fever have an occult fungal infection; thus the empirical strategy necessarily entails treating many to potentially benefit a minority.

The availability of lipid formulations of amphotericin B, azoles with activity against yeasts and moulds (itraconazole and voriconazole) and echinocandins that are active against *Candida* and *Aspergillus* species and have significantly less toxicity than conventional amphotericin B has prompted many centers to use these agents early as prophylaxis rather than later as empirical therapy for neutropenic fever.

In a randomized, double-blind trial of autologous and allogeneic HSCT recipients, micafungin was superior to fluconazole as prophylaxis based on pre-specified criteria that included absence of a breakthrough fungal infection and the absence of modifying the antifungal regimen empirically due to neutropenic fever [85]. The duration of study drug encompassed the neutropenic period, but not the period after neutrophil recovery where GVHD would be expected to occur. The frequency of breakthrough candidemia was similar in both arms, but there was a trend to fewer episodes of invasive aspergillosis in allogeneic HSCT recipients receiving micafungin. The superiority of micafungin was principally driven by a lower frequency of persistent neutropenic fever requiring empirical modification of the antifungal regimen. An unanswered question relates to whether modification of the antifungal regimen is required empirically solely on the basis of persistent neutropenic fever in patients receiving a mould-active drug as prophylaxis.

Two antifungal trials comparing fluconazole with itraconazole have addressed this changing epidemiology by extending the period of administration of antifungal drugs from the time of the conditioning regimen through at least the period corresponding to risk of acute GVHD [86, 87]. Itraconazole was associated with fewer cases of invasive mold infection, but overall survival was similar [86, 87]. Hepatic toxicity and discontinuation because of gastrointestinal intolerance were more common in itraconazole recipients [86]. Itraconazole led to an increase in cyclophosphamide metabolites, which in turn were associated with hyperbilirubinemia and nephrotoxicity during the early transplant period [88]. This finding reinforces a note of caution about itraconazole and newer second-generation triazoles, which are potent inhibitors of cytochrome P450 isoenzymes, with regard to the potential for drug-drug interactions. Voriconazole (compared with fluconazole) is being evaluated as prophylaxis in an ongoing randomized study.

Posaconazole was compared with fluconazole as prophylaxis in allogeneic HSCT recipients with significant GVHD in a prospective, randomized, double-blinded study that enrolled 600 patients [89]. Posaconazole led to a significant reduction in the incidence of invasive aspergillosis, in the total number of invasive fungal infections while on treatment, and in the number of deaths attributed to fungal infection. Both drugs were well-tolerated, and overall survival was similar between the two treatment groups. Assuming these results are confirmed in a peer-reviewed publication, posaconazole should be considered as the gold standard for prophylaxis in allogeneic HSCT recipients with significant GVHD.

The strategy of pre-emptive antifungal therapy does not have standardized, well-defined criteria, but generally involves the use of laboratory markers (e.g., galactomannan, beta-glucan, PCR), radiological monitoring (e.g., chest CT scans), or both, to identify early fungal infection prior to the development of clinically overt disease. Pre-emptive antiviral therapy based on surveillance antigen or PCR detection has become a standard of care for preventing CMV disease in allogeneic HSCT recipients. Pre-emptive antifungal strategies are at an exploratory level and will require validation, ideally in randomized studies. In an open-label, feasibility study, Maertens et al. [90] used serial serum galactomannan and chest CT scanning to detect early aspergillosis in neutropenic patients at high risk for invasive fungal infections who were receiving fluconazole prophylaxis. This strategy reduced the use of empirical antifungal therapy and successfully identified cases of early invasive aspergillosis, but may not be adequate to identify early infection with non-*Aspergillus* moulds.

Empirical antifungal therapy based solely on persistent neutropenic fever may become an outdated concept due to the availability of effective and safer antifungal agents and improved risk stratification methods. An alternative strategy may involve using an agent with activity against yeasts and moulds as prophylaxis in those at highest risk

for invasive mould infections (e.g., prolonged neutropenia following cytotoxic chemotherapy or allogeneic HSCT recipients with significant GVHD). An alternative strategy that merits evaluation in clinical trials involves using a narrower spectrum antifungal agent in combination with pre-specified laboratory markers and chest CT scans to facilitate early detection of breakthrough fungal infection and pre-emptive modification of the antifungal regimen.

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